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SOIL CHARACTERISTICS

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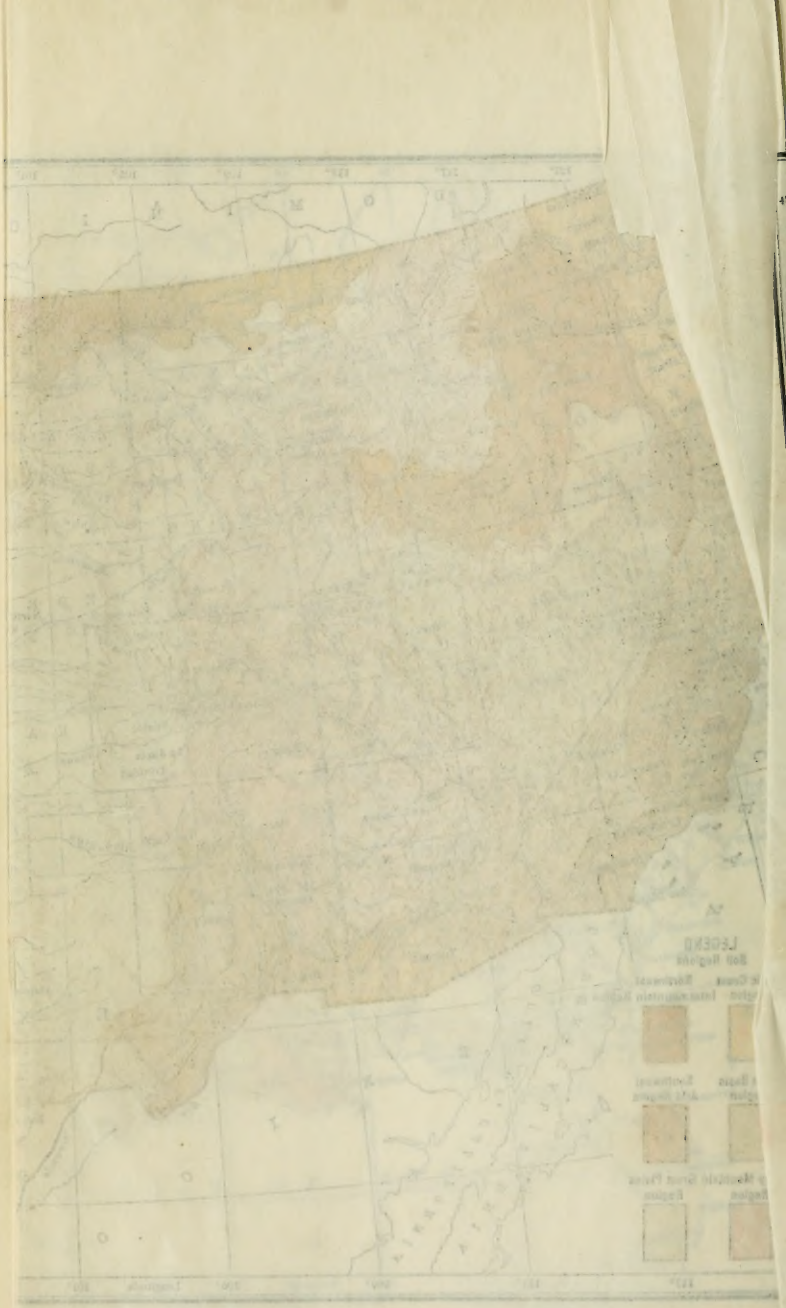
Frontispiece—"Province refers to a large land area in which either the mode or the source of origin, or both, of the soil have been quite similar throughout. . . . The word region is substituted for province in those areas in which the survey has not been extended sufficiently to permit of classification according to any well-defined and common mode or source of origin." Map from Bul. 96, Bureau of Soils, U. S. D. A.



SOIL CHARACTERISTICS

A FIELD AND LABORATORY GUIDE

BY

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PREFACE

Our present soils have been formed by the action of physical, chemical and biological forces, extending over thousands of years. The measurement of the ability of any soil to produce crops must take into consideration all of these forces. Before these forces can be studied in detail, it is necessary that we become acquainted with the soil as it now is, study the outstanding characteristics and then take up those factors that affect its fertility.

This manual is written with this point in view, in the hope that it may serve as a guide to the student, the instructor and the investigator. The material presented has been used by the author, for a number of years, in his investigational and teaching work.

In addition to the references given in the text, frequent use has been made of various manuals and text books dealing with the particular subject matter. In section 3, Soil Fertility, the chemical data presented, follows closely the official A. O. A. C. methods. It will be noticed that practically all references are given to American publications. This was done, without any reflection on the excellent quality of work accomplished abroad, but because the American literature is available to all American institutions, many of which do not have the foreign publications.

Acknowledgement is given for the valuable assistance rendered by Mr. M. S. Anderson of the U. S. Bureau of Soils, Mr. M. Tosterude of the Univ. of Wisc., Mr. G. M. McClure of the Univ. of Ohio, Drs. W. H. Stevenson, R. E. Buchanan and P. E. Brown of Iowa State College and to Mr. C. C. Thomas of the Williams & Wilkins Publishing Co., Baltimore, Md.

Any suggestions in regard to an improvement of the manual will be welcomed.

PAUL EMERSON.

AMES, IOWA.
July, 1925.

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SOIL CHARACTERISTICS

SECTION 1

A GENERAL STUDY OF SOILS

Soil may be defined as *a heterogeneous collection of rock and mineral fragments whose decomposition and disintegration have been brought about by the action of physical, chemical, and biological forces, working singly or in combination.* From the viewpoint of the agriculturist, the main function of the soil is the support of plant growth, hence the definition must be extended to include the following: . . . *and containing sufficient decomposable plant remains to serve as a source of energy in stimulating the action of these forces to produce soluble plant food in an amount sufficient to support the growth of plants commonly used in agricultural pursuits.*

Classification of Soils.—As our present soils are a result of actions that have continued throughout all the ages, it is evident that they would differ markedly in their characteristics. The kind of rock, or rocks, from which they have been formed, and the time, manner, and place of their formation, together with certain features that they now possess, present definite factors that can be correlated, and thus serve as a basis for a definite classification.

Principles of Classification.—An extended study upon a large number of individuals has enabled the botanist to work out a definite system, whereby any plant may be accurately classified. The unit or basis of classification is the *Species.* All the descendents from one stock compose a *Species.* All assemblages of species that have essential parts constructed on the same particular plan, are grouped into *Genera.* All groups of genera that resemble each other in certain particulars are designated as *Orders,* while the orders are still further grouped, in the same manner, into *Classes.* Thus one is enabled to take an individual plant, examine its several parts, and accurately identify it. The plant

is first placed in the correct *Class*, then in the proper *Order*, *Genus*, and *Species*, according to the particular characteristics of the individual.

General Soil Classification.—The general classification of soils is based upon outstanding characteristics, and usually includes large areas. For instance, an area may be classified in a general way, as a humid soil area, but it may contain certain areas within its boundaries that have well-defined characteristics, such as timbered, prairie, etc. This classification is of value in locating the position of soils that have general characteristics, thus serving as a basis for a more detailed classification. Hence soils may be classified variously according to:

1. Temperature:
   a. Tropic.
   b. Temperate.
   c. Frigid.
2. Precipitation:
   a. Arid, rainfall less than 10 inches.
   b. Semiarid, rainfall 10 to 20 inches.
   c. Subhumid, rainfall 20 to 30 inches.
   d. Humid, rainfall more than 30 inches.
   e. Super-humid, saturated or partially saturated soils.
3. Native Vegetation:
   a. Prairie soils; grasses.
   b. Timber soils; trees.
   c. Alkali soils; salt bushes, etc.
   d. Acid soils; sorrel, etc.
   e. Peat and muck soil; reeds, sedges, etc.

4. Lithological Formation: Classified according to the rocks from which they have been derived; granitic soils, etc.

5. Geological Formation: Classified according to the geological forces that have functioned in their formation. Thus we have soils that have been formed in place (sedentary soils) and soils that have been moved (transported soils).

   a. Soils formed in place—Sedentary soils.
      (1) Residual, formed by decomposition and disintegration.
      (2) Cumulose—swamp and marsh soils.
   b. Soils moved.—Transported soils.
      (1) Colluvial, formed by gravity.
      (2) Water formation.

(*a*) Marine—sea laid.
(*b*) Lacustrine—lake laid.
(*c*) Alluvial—stream laid.
(3) Glacial, ice formed.
(4) Loessal, wind formed.

Systems of Soil Classification.—The Russian system of soil classification is the oldest and most elaborate of all systems. The divisions are made on the basis of climate, the processes of soil formation and the physical, chemical and biological processes of the soil itself. The rocks from which the soil is derived only receive secondary consideration. The French system is based entirely on the geological formation. The soils of a certain geological area are classified according to their mechanical and chemical composition. The systems of other countries combine various features of these two.

THE DECIMAL SYSTEM OF CLASSIFICATION

A decimal system of classification is in use in Illinois,[4] in which the whole number represents the subprovince, the series and the class, and the decimal represents the phases (position of the classes in respect to each other). The system is called the "Illinois System of Classification." To illustrate: a soil has the number 725.1. The number 7 means that it occurs in the Iowan glaciation, the 25 that it is black silt loam, and the 0.1 that clay is found less than 30 inches below the surface.

Soil Classification Similar to Botanical Classification.—The system used in the classification of the different soil areas is analogous to the use of species, genus, order, and class, by the botanist, and differs only in the names used to designate the different groupings. (1) The unit of soil classification is the *Type*, which may be likened to the plant *Species* in that the designation of the *Type* is based upon all the minor details that impart a certain individual characteristic to an area of soil in a certain position. (2) The soil of one area may be related to the soil of another area from the standpoint of the size and arrangement of the different particles, hence we may have a grouping of the *Types* on this basis. This grouping is designated as a *Soil Class*, which might be likened to a plant *Genus*. (3) All soils have a color and this factor is taken as a basis for the assembling of *Soil Classes* and *Soil Types* into the next grouping, the *Soil*

Series. (4) From this point a grouping of the different *Soil Series* on the basis of their origin and formation gives the largest unit of soil classification, the *Soil Province.* (5) Certain great areas in the western part of the United States have not been studied in sufficient detail to classify them as Provinces, hence we have areas that may be definitely classified in the future. These areas are called *Soil Regions.*

Identification and Classification of a Soil Dependent upon Many Observations.—If the botanist is given a complete plant, together with the location from which the specimen was secured, he is able to pick out all those characteristics that enable him properly to classify the individual. In the case of an individual soil sample, the essential characteristics for classification are not present. These characteristics are certain detailed features of an area, impossible to secure in an individual sample. These characteristics must be noted at the time the sample is taken, hence it is impossible properly to classify an individual sample of soil unless all topographical features, necessary for its identification, are supplied in the form of extended notes.

THE UNITED STATES BUREAU OF SOILS CLASSIFICATION

The United States Bureau of Soils classifies soil[2] areas as follows:

1. Soil Regions.
2. Soil Provinces.
3. Soil Series.
4. Soil Class.
5. Soil Type.

1. Soil Region.—A soil region is a great area that has the same general physiographic expression and may or may not have been formed by a predominating group of forces, or by one force. It has not been studied in sufficient detail to classify it properly. It is differentiated mainly on geographic features, hence is a potential field for classification into one or more Soil Provinces.

2. Soil Province.—"A soil province is an area having the same general physiographic expression, in which the soils have been produced by a common force, or by a group of forces." The soils of the area are characterized by the fact that each kind of rock or mineral has yielded a definite result according to the force that has predominated in their formation. "The soil province is named in accordance with some generally accepted

terminology for the area represented, or according to the process by which its soil material was formed."

As a soil region and a soil province differ mainly in geographic features, rather than in soil characteristics, the soils in each are further classified according to those essentials which form a basis for the determination of the *Series, Class* and *Type.*

3. Soil Series.—"A soil series may be defined as a group of soils having the same range in color, the same character of subsoil, particularly as regards color and structure, broadly the same type of relief and drainage and a common or similar origin." "A soil series is named from some town, village, county, or natural feature existing in the area when it was first encountered."

The Color of Soils.—Soils are of various colors ranging from almost pure black to almost pure white, with all shades and variations in between. The range of colors is not confined to the surface. The color of the subsurface and the subsoil may be entirely different from that of the surface. Humid soils rarely are of the same general color throughout the 3-foot section. Arid soils may be quite uniform to a depth of several feet. In the humid soils the surface is usually darker than the deeper levels. The color is also affected by the degree of natural mixing. In some cases the colors are well blended while in others they are imperfectly mixed and the sample will present a mottled appearance. There is at present no standard for color comparison, and one must be governed by his own individual interpretation of color descriptions of known types. A committee of the American Association of Soil Survey Workers is at present working on soil color and the adoption of standard colors and color nomenclature is hoped for at an early date.

Color of Moist Soils.—It is quite generally recognized that moisture exerts a very important influence on soil color. The depth of color is apparently increased with increases of moisture, up to a certain point. The dark-colored soils usually show a greater change with moisture variations than light-colored soils.

Color of Dry Soils.—Soils in an air-dry condition are known to be lighter in color than when wet. Consequently, the color of the soil should be carefully noted at the time the soil is studied, due recognition being given to the moisture content.

4. Soil Class.—The soil class is a grouping of soils on the basis of the same general texture or the same-sized particles. "A

soil class, therefore, includes all soils of the same texture, such as sands, clays, loams, etc. A soil class is not limited in its occurrence to a soil province, but the same class occurs in all provinces or regions." "The class name is wholly descriptive."

Soil Texture.—The texture of the soil refers to the size of the particle. As soils are a mixture of the different-sized particles, the percentage composition of the sample can only be determined by a mechanical analysis. There are, however, certain outstanding characteristics of soils which enable one to judge of their texture, or the predominance of particles of a certain size, by feeling them and studying their appearance.

Sands are quickly classified by their appearance, their feel, and their lack of cohesion, or ability to hold together. A large amount of sand in the soil sample may be detected by becoming acquainted with the feel.

Clays are very fine, hence have a smooth feel. They possess marked properties of cohesion, and moist samples may be rolled between the thumb and forefinger into characteristic spindles. A clay will also take a polish if rubbed between the fingers.

Loams are characterized by their loose, friable feel. Cohesion is not so marked as in clay, yet not entirely absent as in sand. If pressed into a ball and dropped, loams will break more readily than clay, if each sample is of the same degree of moisture.

Sandy loams are detected by their general loamy characteristics and the feel of sand in them.

Silts are characterized by the ability of the particles to slip over each other, thus imparting a more or less greasy feel. Silts will also take a polish when removed from the auger or when rubbed with the thumb nail.

The Size of Soil Particles.—The different soil particles are designated according to size as follows.

Stones over 32 millimeters.
Gravel 32 to 2.0 millimeters.
Very coarse sand 2.0 to 1.0 millimeters.
Coarse sand 1.0 to 0.5 millimeter.
Medium sand 0.5 to 0.25 millimeter.
Fine sand 0.25 to 0.1 millimeter.
Very fine sand 0.1 to 0.05 millimeter.
Silt 0.05 to 0.005 millimeter.

As a soil is usually made up of a mixture of different-sized particles, the designation of the specific class depends upon the

amount or per cent of each of the various sizes. The United States Bureau of Soils recognizes the following classes:

Peats: consisting of 35 per cent or more of organic matter, sometimes mixed with more or less sand or soil.

Peaty Loams: a large quantity of sand and silt and a little clay mixed with 15 to 35 per cent organic matter.

Mucks: a large quantity of clay and some silt mixed with 25 to 35 per cent of partly decomposed organic matter.

Clays: soils with more than 30 per cent clay, usually mixed with much silt; always more than 50 per cent silt and clay.

Silty Clay Loams: 20 to 30 per cent clay and more than 50 per cent silt.

Clay Loams: less than 50 per cent silt and some sand and 20 to 30 per cent clay.

Silt Loams: more than 50 per cent silt mixed with some sand and 20 per cent clay.

Loams: less than 20 per cent clay and less than 50 per cent silt and from 30 to 50 per cent sand.

Sandy Clays: small amounts of clay up to 30 per cent and 20 per cent silt.

Fine Sandy Loams: more than 50 per cent fine sand and very fine sand mixed with less than 25 per cent very coarse sand, coarse sand and medium sand, much silt and a little clay; silt and clay 20 to 50 per cent.

Sandy Loams: more than 25 per cent very coarse, coarse and medium sand; silt and clay 20 to 50 per cent.

Very Fine Sand: more than 50 per cent fine sand and less than 25 per cent very coarse, coarse and medium sand, less than 20 per cent silt and clay.

Fine Sand: more than 50 per cent fine sand and less than 25 per cent very coarse, coarse and medium sand, more than 20 per cent silt and clay.

Sand: more than 25 per cent very coarse, coarse and medium sand, less than 50 per cent fine sand, more than 20 per cent silt and clay.

Coarse Sand: more than 25 per cent very coarse, coarse and medium sand, less than 50 per cent of other grades, less than 20 per cent silt and clay.

Gravelly Loams: much sand and some silt and 25 to 50 per cent very coarse sand.

Gravels: more than 50 per cent very coarse sand.

Stony Loams: a large number of stones over 1 inch in diameter.

5. Soil Type.—The soil type is the unit or individual of an area. It connotes all the features of the class, series, and province, hence is limited to a single class, a single series, and a single province. It "is a soil which throughout the area of its occurrence has the same texture, color, structure, character of subsoil, general topography, process of derivation, and usually derivation from the same material."

Naming the Type.—The name of a soil type consists of two or more words. The first word is the series name and the second is the class name. When there are more than two, the

extra words are always explanatory, for example, Webster loam, Miami fine sandy loam, Sarpy very fine sand, etc. Occasionally the relation of the type to certain outstanding physiographic characteristics is expressed as phases, for example, Carrington loam (steep phase); Tama silt loam (rolling phase) etc.

In studying the factors that are taken as a basis for naming the type the following characteristics are considered more or less in detail:

In the Field:

General consideration;

1. Origin. Geological formation, usually determined from geological or reconnaissance survey reports, residual, cumulose, glacial, eölial, alluvial, etc.

2. Native vegetation. Almost impossible to determine in many areas. Mainly limited to prairie or timber areas.

3. Topography. General topographical features of the surface area or the lay of the land.

4. Natural drainage. The kind of drainage whether efficient or not. Geological age of the drainage systems. Many streams were once quite broad, hence we have different types of terrace soils.

5. Organic matter. Generally estimated by color of soil.

6. Agricultural value. Productive capacity.

Specific considerations;

7. Profile. Changes in soil at various depths, usually,
   a. 0 to 12 inches. Surface.
   b. 12 to 24 inches. Subsurface.
   c. 24 to 36 inches. Subsoil.
   d. depths where change in color, structure or texture occurs.

8. Color of profile at different depths.

9. Structure or granulation of profile at different depths. Texture, porosity, friability, plasticity, etc.

10. Physical composition (class of soil) of profile at different depths. An estimation of percentage composition of the different-sized particles.

In the Laboratory:

11. Reaction.—Acid, neutral or basic. Sometimes determined in the field.

12. Chemical analysis.—Plant food content, analysis of various elements.

When students are making a study of soils for the first time, the soils should be designated by number as described in "The Decimal System of Classification" and full notes taken at the time of sampling. The class should be determined as accurately as possible. In case the class cannot be accurately determined in the field, reserve the sample for a mechanical analysis to be made later. No attempt should be made to name the type until thorough familiarity with the soil series, the soil class, and those laboratory and field factors that serve as a base for the naming of the type, is acquired. As the student becomes more proficient and larger areas are to be studied, he should consult the state soil survey reports. From these reports he can work out a key, showing the principal topographic divisions, the geological formation and other features, that will be of great assistance in his field studies.

THE SOIL SURVEY[3]

The purpose of a soil survey is to define, identify, map, classify, correlate, and describe soils. It is practically impossible to give definite directions for the making of a soil survey. The art is learned best through contact or serving an apprenticeship with an experienced party. The student, however, may readily learn the fundamental principles underlying the soil survey and become proficient in the determination of soil type by a close study of soils in both the field and laboratory, and thus correlate the importance of type with crop production, as will be brought out later. The problem is more or less simplified by the fact that the predominating types in any vicinity do not usually exceed 15 or 20.

The Detailed Soil Survey.—A detailed soil survey of any area (usually a certain county of a state) in a province consists of:

1. The preparation of an accurate base map.
2. The determination of the different soils series within this area.
3. The identification of the various classes and types of soil by a close study of the field samples and marking the border of the various types upon the map.
4. The preparation of an accurate soils map showing the position of all types, and a report of agricultural conditions.

Making the Survey.—It is practically impossible for students to make a detailed soil survey over a large area. They may, however, study a small area in exactly the same manner as a large area would be surveyed. In order to do this, the characteristics that form the basis of a soil series, and determine in general the class and type, should be studied in detail in the laboratory, giving special emphasis to those soils that will be encountered later in the field.

The Field.—The field should be an area containing five or more soil types. In Iowa a number of farms equivalent to the area of a section (one square mile) is selected for study. In sections where there is a wide variation between types, the size of an area may be reduced. The area should include enough types to acquaint the students thoroughly with the great variability, existing between types, and also give them some idea of the relationship existing between various types and crop production.

Equipment.—The material required for making the survey and taking samples for future laboratory determination are: (1) the base map upon which will be located the boundaries of the various soil types. (2) An auger, trowel, spade, or post-hole digger. The auger may be used for locating the type boundaries. The other tools are used in securing the samples. (3) Three sampling cloths, 24 × 24 inches or three pans that will hold about 2 quarts each. The best sampling cloths are cut from 48-inch, white oil cloth. (4) Small sacks or jars for holding the composite sample. (Never place the samples in paper bags.)

Preparation of Base Map.—Draw to scale, of not less than 500 and preferably 100 to 200 feet per inch, an outline map of the field to be studied. Show the boundaries of all the fields, the locations of all gates, roads, trees (when isolated in the fields) orchards, wooded areas, meadows, pastures, cultivated fields, buildings, and the locations and directions of all ditches, slopes, tile-drains, etc. The best maps are made by using a plane table, taking the measurements with a tape. For rough work, however, the distances may be approximated by pacing and the angles determined by sighting along a card or pencil, the paper being placed on the top of some convenient fence post. If it is desired to use the plane table, the instructor is referred to any surveying textbook for directions as to its use.

The map is best prepared on coordinate paper which should be of heavy quality and plainly marked. The markings should

be heavy lines 1 inch apart arranged in squares with the space between divided into 10 equal portions indicated by lighter lines. This map is to be used later for locating the boundaries of the soil types, for locating the positions from which samples are taken, and for the preparation of the soils map to be turned in with the written report at the end of the course.

History of Area.—The agricultural history of each area to be surveyed should be secured. Interview the owner, or the tenant and secure as far as possible the cropping systems followed, the methods of tillage, the treatments such as manures, green manures, lime, fertilizers, etc., that have been applied and the relative amounts. Secure the yields, either approximate or exact. Have this data extend over as many years as possible. While this information will not be of any value in determining the soil type, further than to show that certain types may be related to crop production, it will be of value if the samples secured are used as a basis of analysis in the fertility determinations.

The Soil Profile.—Humid soils are usually studied to a depth of 40 inches, arid to a depth of 6 feet or more. The best way of making the study would be to dig a large hole to the desired depth and carefully examine the sides, much as one would examine the face or profile of a cliff. As this method requires too much time and labor, the different soil layers (soil profile) are reconstructed by means of an examination of borings taken from the various depths.

The Color of the Profile.—The color of the soil profile is usually made up of two parts, the color of the surface material and the color of the subsurface material. Under humid conditions there is usually a marked difference between the two, and sometimes the subsoil may have different colored layers. Furthermore the depth of change of the different colors will vary with different locations.

The Texture of the Soil Profile.—The texture of the surface portion of the soil profile may be influenced by varying amounts of organic matter, and extreme care must be used in judging the texture of the mineral portion. Also the texture of the lower levels is usually markedly different from that of the surface. Generally the surface soil is lighter in texture, that is, it has less clay and more sand than the subsoil. If there is a change in the texture in the first few inches it is usually neglected as it may soon

be changed by cultivation. However, when a change in texture occurs at the depth of the plow line it must be carefully considered. In general the class to which a soil type belongs is defined by the texture of the surface soil to the depth of the plow line.

The Structure of the Soil Profile.—The structure of the soil has a marked influence upon the water-holding capacity, the drainage, the tillage operations, and the general ability of the soil to produce crops. It is important to know whether the material is loose, friable, and porous, or whether it is compact and impervious. If any portion of the profile is a clay, careful note should be made as to whether it is friable, plastic, or stiff. Some loams have a floury appearance while others have a compact and impervious structure. Some soils may be quite sticky while others of the same or finer degree of texture may not exhibit the sticky characteristic. Some clays are smooth and unctuous while others are quite gritty. Some soils, especially those derived from micaceous rocks may have a smooth greasy feel.

Careful notes should be made of all variations in color, texture, and structure of the soil profile in order that a sound basis may be had for naming the type.

Study of the Soil Profile.—Make borings at 50-foot intervals, using a 1½-inch auger fitted with a 36-inch handle marked in 6 inch intervals, and examine the soil closely at the following depths, 0 to 12 inches, 12 to 24 inches, and 24 to 36 inches. The exact depth for each examination will depend upon the nature and character of the soil being investigated. Note carefully any difference as regards texture or color in either the surface, the subsurface or the subsoil. A change in the character of the soil at any depth indicates the possibility of a difference in type, therefore it becomes necessary to establish the boundaries in depths between the different types whenever they are found.

Borings taken from the different depths at one station should be compared with those secured from other stations and any changes noted. Frequently the gradation between soil types is so slight that it is almost impossible to detect a change without a direct comparison. This makes it difficult, when the scale of the map is small, to establish the boundaries between types with any degree of accuracy. If, however, there is a distinct change between the sample taken at one station and that taken at another, the location of the boundaries of the types should be determined by making the borings at shorter intervals.

Mark the position of the various types of soil on the base map, designating each by number or if possible by name. Secure samples of each for further study and classification in the laboratory where they may be compared, at leisure, with known types. The samples taken at this time are for preliminary study only. When the area has been surveyed, the location of the boundaries of all types definitely determined, composite samples should be secured for laboratory studies. The map will be of great assistance in determining the positions from which these samples are to be drawn.

Soil Samples.—Composite samples are secured from a number of individual samplings (at least 10) taken at regular intervals from representative portions of the type. All unusual situations as paths, places where manure piles have stood, brush heaps have burned, ditch banks, clumps of bushes, etc., should be avoided. The samples are named according to the depths at which they are taken. The depth will vary according to the different factors as geological formation, method or cultivation, etc. If the soil is a deep loess, peat, etc., its appearance will be uniform throughout and the depth chosen must be arbitrary, for instance, 0 to 12, 12 to 24, and 24 to 36 inches. If, however, there is quite a variation between the underlying strata, the samples should be taken to the depth at which a marked change occurs. The most common practice is to take the surface sample to the depth of the plow line, approximately 7 inches, the subsurface sample from the plow line to 20 inches, and the subsoil samples 20 to 36 or 40 inches or deeper. If the surface sample is taken to a depth of $6\frac{2}{3}$ inches, and the others to 20 and 40 inches, the weight per acre of soil for each depth will be approximately two, four and six million pounds.

Amount of Sample.—The amount of sample to be taken depends entirely upon the uses to which the soils are to be subjected. If fertility studies only are to be made, 1 quart will be sufficient, while if greenhouse studies are to be made, the amount may be several tons. An 8-quart sample will usually be sufficient for both the physical and fertility tests. Bacteriological samples should always be taken fresh, and used immediately (see Sec. 4, Soil Microbiology, page 174).

Securing the Sample.—Too much emphasis cannot be given to the care necessary in taking soil samples. The samples may be taken with a soil auger, trowel, post-hole digger or spade, according to the amount required. The auger or trowel is used

mainly for the samples that are intended for laboratory use. All refuse material such as dried grass, roots, etc., should be removed from the surface preparatory to taking the sample. In case an auger is used, the soil should be pressed firmly over the spot where the borings are to be made.

Taking the Surface Sample.—In taking the surface sample the first boring should be approximately one-half or two-thirds the desired depth. The feet should be held close to the auger when withdrawing it, in order to prevent the removal of a large lump of the surface soil. When a clean hole is established, the boring is made to the desired depth and the hole cleaned out by succesive borings before proceeding to the deeper levels. In case a trowel, or a spade, is used, a hole with one perpendicular side is made to the desired depth and a thin slice of soil of equal thickness, from top to bottom, is taken as the sample from the perpendicular side. Only a small amount of soil is taken from each place, but *it must represent accurately the true condition of that soil for the depth at which it is taken.* The sample for each depth is placed in a suitable container or on a sampling cloth and the samplings from the other locations added to the corresponding sample.

Taking the Subsurface Sample.—The subsurface sample is taken immediately below the spot where the surface sample has been secured. The depth will vary according to the depth of the surface sampling. In case the surface sample was taken to a depth of $6^2/_3$ inches, the subsurface sample is usually taken to a depth of 20 inches; if the surface sample was to 12 inches the subsurface is taken to 24 inches. If the auger is used, all loose soil must be removed from the hole before proceeding and all questionable soil at the top of the core must be discarded when the auger is withdrawn from the lower levels. If the trowel or spade is used it will be necessary to remove all contaminating surface soil from the hole before proceeding to the lower levels, this being best accomplished by simply enlarging the hole. The post-hole digger is rarely used below the depth of the surface sample except in those cases where a large sample is desired. The subsurface sample is placed in a separate container from that used for the surface sample.

Taking the Subsoil Sample.—The subsoil sample is taken in the same manner as the other samples, using the same precautions. The depth varies from 20 to 48 inches or deeper.

When the first set of samples has been secured, the entire equipment is moved to the new location and the sampling process repeated. Each depth of sampling is placed in its respective container.

Mixing the Sample.—When the total number of individual samples (not less than 5, and preferably 10 or more, depending upon the size and variability of the area to be sampled) for the particular type has been made, the samplings for each depth are thoroughly mixed. The mixing may be done in the container in which they have been placed, but more satisfactory results are secured by the use of a mixing cloth. Pile the samples in the center of the cloth and, digging from the bottom of the pile, spread the soil evenly over the cloth. Remove all sticks and stones. Fold the cloth in such a manner that the soil is again piled in the center. Again spread out as before and repeat the process at least three times. Again pile in the center, cut the pile in half, and discard one portion. Spread and repile again three times and discard one-half as before. Repeat the mixing and discarding process until the remaining portion is the size of the desired sample. This sample, if properly prepared, contains an equal amount of soil from each of the individual samplings, and is therefore a truly representative (composite) sample of the area from which it is taken. This composite sample should be placed in a suitable container, cloth sack or jar, never in a paper bag, and labeled.

THE MECHANICAL ANALYSIS OF SOILS

While an experienced person may tell, by a close examination and by the feel of a soil, its approximate composition, and be able to classify it readily as a loam, sandy loam, fine sand, etc., the inexperienced person cannot do this. He may be able to classify those soils that have outstanding characteristics but cannot do more than guess at the composition of many of the types that are found. The only way that he can be sure of the proper classification is to make a mechanical analysis and determine the amount of the different-sized particles.

Preparation of Sample for Mechanical Analysis.—Dry the sample at 105°C. until free of all hygroscopic moisture, or determine the hygroscopic moisture content (see Sec. 2, page 27) and deduct from the final result. Grind with a rubber pestle until all concretions are broken up. Prepare at least 500 grams.

Determination of Sand and Gravel.—The per cent of gravel and practically all grades of sand are determined by the use of sieves with openings of the required size for making the necessary separations. Special sieves are now manufactured for this purpose and may be purchased from any chemical supply house. The usual types are made in nests of five with openings of 20, 40, 60, 80, and 100 meshes per inch, the size of the opening depending on the size of the wire used in making the mesh. The size of the opening may be determined: (1) by measuring the wire, determining the number of wires per unit length, and calculating the space between or, (2) by measuring the size of the opening directly by the microscope (see Measurement of Soil Particles, page 19).

The sample is passed through a series of sieves beginning with the coarser mesh. Gently shake the sieves and jar by bumping against the hand, but never try to rub the soil through the sieve. (If a large number of samples are to be determined, a mechanical shaker will be of great help.) The largest particles are removed first, the next largest are in the second sieve, and so on through the series. The different separations are weighed and the percentages calculated from the total weight of the sample used in the beginning. If great accuracy is desired the separations are made by washing the material through with water. The very fine sand, the silt and the clay cannot be separated accurately by the sieve method.

Determination of Fine Sand, Silt, and Clay.—The separation and determination of the size of the various soil particles are based on the fact that the larger particles settle in solution at a faster rate than the smaller particles. The separations cannot be completed in the first trials due to the fact that the larger particles carry some of the smaller down with them. A repetition of the process will, if repeated enough times, completely and accurately separate to any degree of size. The determinations may be made on the original samples. Better results are secured if they are made on the residual soil left from the determination of the gravel and coarser particles of sand.

Subsidence Method.—Weigh out four 10-gram portions of air-dry soil, place two in tared crucibles, ignite and determine the per cent loss on ignition. Place the other two in shaker bottles and add 5 to 10 cubic centimeters concentrated ammonia and 150 to 200 cubic centimeters distilled water. $\frac{N}{5}$ HCl may be used,

instead of ammonia, to overcome any possible digestive effect of the ammonia. Place the bottles in a shaker and agitate until a microscopic examination, of a "bacteriological loopful" of the solution, shows that the separation is complete, *i.e.*, no granules or irregular shapes appear in the field. The length of time will vary widely, sometimes requiring as much as 24 hours to complete. Transfer the soil and water from the shaker bottles to 2-quart milk bottles, carefully washing into the milk bottles all adhering particles from bottles and stoppers. Cover the bottles with an inverted two-holed rubber stopper. Place in one hole a short bent tube, extending a short distance into the flask and place in the other hole, a long tube that reaches nearly to the bottom of the bottle. The lower end of this tube is bent suddenly upward, upon itself like an U, one side of which is many times longer than the other, and is cut off so that the end will be square and parallel with the bottom of the bottle. Adjust this tube so that the opening will be 1½ inches from the bottom of the bottle. Make a mark on the bottle 3 inches from the bottom and fill to this mark with a jet of water, driven in with sufficient force to stir up the contents thoroughly.

After the liquid has stood long enough for the largest particles to settle below the end of the tube, as determined by microscopic examination, the liquid containing the most of the smaller particles is blown off into a beaker, placed at the free end of the long tube. The process of filling, settling, and blowing off is repeated until the grades that settle are free of the smaller particles of silt and clay. The larger particles are now separated by shortening the settling period.

The water containing the silt and clay is poured into a large bottle, thoroughly shaken, an aliquot taken, evaporated to dryness, placed in a tared crucible, weighed, ignited, weighed again, and the total amount of clay and silt determined together.

The water containing the separated coarser particles is decanted, separated by sedimentation, and each grade freed of the water by evaporation. Each grade is placed in a weighed crucible, dried, weighed, ignited, and again weighed and the per cent determined.

The silt and clay can only be separated from each other by increasing the time of settling, but the process involves a long period. As some of the particles will stay in suspension for months, their separation is more quickly effected by centrifuging

the suspension for varying lengths of time and measuring the soil particles by means of the microscope.

SOIL COLLOIDS

Certain soils contain extremely fine particles that are not thrown down in the centrifuge, but remain in suspension under all conditions of force. These particles have little importance from the standpoint of the determination of the separates, but they have a very important rôle to play in the structure of soils and in imparting certain properties, especially that of adsorption (see Adsorption, Sec. 2, page 42).

United States Department of Agriculture Method of Isolating Soil Colloids.[1]—Agitate 100 pounds of soil with 500 pounds of water in a barrel churn for 2 hours. Allow to stand 18 hours and siphon off the turbid liquid. Repeat the treatment 3 times or until the soil fails to give any appreciable quantity of suspended matter. In case the second treatment gives a heavier suspension than the first treatment, use water containing 1 part of ammonia to 3,000 parts of water. The turbid extract from the soil is now run through a high-power centrifuge (supercentrifuge) driven at the rate of 17,000 revolutions per minute. In this process, the particles are exposed to a force of approximately 17,000 times gravity for about 3 minutes. The colloidal suspension which passes through the centrifuge is concentrated by forcing the water through a battery of Chamberland-Pasteur filters (bougie F), the colloidal matter collecting on the outside of the filters. The slimy film of colloid which collects on the outside of the filter is readily removed by detaching the bougie and blowing air into it. The colloidal material is reduced to an air-dry condition on the steam bath. It is practically impossible to measure the size of these particles by microscopic means. Their presence is indicated, in most cases, by the use of the ultra microscope (dark-field illuminator).

Suggested Experiments

Estimation of Gravel, Sand, and Silt. Fill with soil a 250-cubic centimeter-graduated cylinder to the 100-cubic centimeter mark, compact by dropping on the open palm 3 or 4 times and fill to the mark. Add 50 cubic centimeters ammonia water and sufficient distilled water to make a total volume of 200 cubic centimeters. Stopper the end of the cylinder and shake vigorously for 5 minutes. Allow to settle, preferably overnight, and determine by inspection the line of demarcation between the different

grades of soil particles. The approximate per cent of each grade is read directly on the graduations of the cylinder.

Estimation of Sand, Silt, and Clay.—Place 100 grams of soil in shaker bottles, add 75 cubic centimeters of water and 1 cubic centimeter of ammonia and shake several hours. Wash contents into a 250-cubic centimeter beaker (marked No. 1) and add 100 cubic centimeters of water. Mark the height of the water, stir thoroughly, and allow to settle 1 minute. Pour the supernatant liquid into beaker No. 2. Fill beaker No. 1 to the mark. Stir both Nos. 1 and 2 and allow to settle 1 minute. Pour the supernatant liquid from both into a large beaker marked No. 3. Wash the sediment from beaker No. 2 into No. 1, stir thoroughly, and add the supernatant liquid to beaker No. 3. The sand is now in beaker No. 1, the silt and clay in beaker No. 3. Separate the silt and clay in exactly the same manner as the sand was separated, allowing 30 minutes for settling. Evaporate the excess water and determine, by weight, the per cent of the various-sized particles.

THE PREPARATION OF SOIL FRACTIONS

The mechanical analysis shows the different sizes of the soil particles but it gives no indication of the influence of these particles on the various physical phenomena of the soil. In order that these features may be studied later, and that the student may check his results by making a gross analysis, the preparation of soil fractions is included here. The students should work in groups.

An amount of soil equivalent to 1,000 grams in the dry state is prepared as for mechanical analysis, except that no deflocculation agent (ammonia or hydrochloric acid) is added. A rubber pestle is used to break up the adhering particles. The separations are made by settlings and decantations. As it will be almost impossible to separate the clay by these means, the latter is separated from the water in which it is suspended by the use of an ordinary cream separator. This is accomplished by allowing only a small stream to enter the separator and at the same time keep the speed relatively high (8 to 10,000 revolutions of the bowl per minute). The clay will be deposited on the sides of the bowl or on the plates of the separator which are dried and the clay scraped off. The fractions are weighed, placed in separate containers, labeled and stored for physical determinations. These fractions are particularly valuable in the adsorption phenomena determinations, also in the water movement determinations given in Section 2.

THE MEASUREMENT OF SOIL PARTICLES

The size of the individual particles and their arrangement has a marked influence upon the physical properties of the soil. Due

to the fineness of the particles making up soils it is impossible to determine the size by any other means than microscopic measurement. The use of the microscope, however, is not limited to measurement. The weight of the soil particles, which are more or less spherical, and to a certain extent their chemical composition, may be determined. The student is referred to the field of chemical microscopy for details in this respect.

Unit of Length.—The unit of length used in microscopic measurement is the micron (designated by the symbol μ) which is one-thousandth part of a millimeter (0.001 mm.) or (0.00003937 inch). The art of microscopic measurement is called "Micrometry" and the attachments by which the measurements are made are called "Micrometers." There are 4 kinds of micrometers in use: the stage, the eyepiece, the filar, and the step. The first three are in common use.

The Stage Micrometer.—The stage micrometer is a slide with a scale engraved on it divided into hundreths of a millimeter (0.01) every tenth line being made longer than the intervening ones to facilitate counting. This forms the basis of measurements to be made by the other means. In other words, the stage micrometer is used for calibrating the microscope and must be withdrawn in order that the object to be measured may be put in its place.

Method of Measurement with the Camera Lucida.—Attach a camera lucida to the eyepiece of the microscope and adjust the stage micrometer upon the stage of the microscope and accurately focus the divisions of the scale.

Project the scale of the stage micrometer on a piece of paper and with a pen or sharp pencil, sketch in the magnified image. Mark on the paper the optic combination, (ocular, objective, tube length, and angle of the mirror) employed to produce this particular magnification. The measurement of the desired object is accomplished by substituting the object for the stage micrometer and projecting the image of the object upon the scale. It is necessary to make a new scale for each of the possible combinations of objectives and eyepieces, also for each change of tube length. The student should work out such a set for future determinations if this apparatus is to be used. It must be remembered that the scale drawn on the paper is merely a magnification of the stage micrometer thus representing 0.01 millimeter for each division.

The Eyepiece Micrometer.—The eyepiece micrometer is by far the most satisfactory measuring device for general use as it is kept within the eyepiece and does not interfere with the usual manipulations of the microscope. It is a glass disc that is inserted in the ocular. The surface of the disc is etched with a scale or with squares divided to tenths of a millimeter (0.1 mm.).

Method of Measurement with the Eyepiece Micrometer.—Insert the disc in the ocular, place the stage micrometer on the stage and focus on the scale of the stage micrometer. The lines of the eyepiece will appear in the same plane. Make the lines of the two micrometers parallel to each other. Increase or diminish the length of the tube until two of the lines of the eyepiece micrometer coincide with those bounding one division of the stage micrometer. Carefully note the number of included divisions. Calculate the value of each division of the eyepiece micrometer, in terms of the stage micrometer, by the formula: $X = 10\ Y$, when X = the number of included divisions of the eyepiece micrometer and Y = the number of included divisions of the stage micrometer.

The value of each set of combinations of objectives and eyepieces must be worked out.

The measurement of the desired object is accomplished by substituting the object for the stage micrometer and reading off the number of divisions of the eyepiece micrometer that it occupies. The result may be expressed in millimeters or microns by applying the standard value for the optical combination used.

The Filar Micrometer.—The filar micrometer is an ocular having a fixed wire stretched horizontally across the field with a vertical reference wire adjusted at right angles to the first, and a fine wire, parallel to the reference wire, which can be moved across the field by the action of a micrometer screw. The trap head is divided into 100 parts, which successively pass a fixed index as the head is turned. A fixed comb, with the intervals between its teeth corresponding to one complete revolution of the screw head, is seen in the field of vision.

Method of Measurement with the Filar Micrometer.—Place the stage micrometer in position on the stage and adjust the filar micrometer until the movable wire coincides with the fixed one and the index marks zero on the trap head. Focus the lines of the two micrometers and adjust them in parallel. Turn the

micrometer screw until the movable line has traversed one division of the stage micrometer. Note the number of complete revolutions, by means of the recording comb, and the fraction of revolutions, by means of the trap head, which are required to make a measurement of 0.01 millimeter. Record the micrometer value in terms of millimeters or microns. The value of each objective must be worked out. Substitute the object for the stage micrometer and measure as in the other methods.

SOIL-FORMING MINERALS AND ROCKS

The soils student should become acquainted with the common soil-forming minerals and rocks. If possible, this information should be secured through a study of Agricultural Geology. If such a course is not available, an exhibit of minerals and rocks should be arranged and the student required to become acquainted with the name and composition of each. The number of specimens should be arranged to correspond as nearly as possible with the rocks and minerals from which the local soils are derived.

References

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2. Marbut, C. F., Bennett, H. H., Lapham, J. E., and Lapham, M. H., "Soils of the United States" (ed., 1913). *U. S. Dept. of Agr., Bureau of Soils, Bull.* 96, 791 pp., 1913.
3. U. S. Dept. of *Agr., Bureau of Soils.* "Instructions to Field Parties," 124 pp., Washington, D. C., 1914.
4. *University of Illinois, Agr. Exp. Sta., Soil Report* Nos. 19, 20, 21 and subsequent issues, 1921.

SECTION 2

SOIL PHYSICS

Soil physics is the interpretation of various soil phenomena by physical methods. The physical properties of soils are not always clearly defined, due to the fact that they are so closely associated with the chemical and biological properties. This is especially true if the soil is considered primarily as a medium for the growth of plants. As plants require moisture, temperature, and air for their best growth, and as these factors are closely correlated with the physical forces, the influence of these factors will govern the order in which they will be taken up.

The Soil Sample.—The various types of soils mapped and described in Section 1 and the soil fractions prepared from one of these soils will serve as a basis for the study of the physical properties of these samples. The samples should include: (1) a sandy type; (2) a silty-loam type; (3) a clay or clay-loam type; and (4) a muck or peat.

Large samples of the fresh soil may be satisfactorily stored in 2-quart mason jars at the time the samplings are made in the work of Sec. 1. Fresh field samples taken at the various depths are always to be used if the weather permits.

SOIL VOLUME AND WEIGHT

The soil is not a solid mass but is made up of many small particles. The size and arrangement of these particles, determine in a great measure the character or tilth of the soil.

APPARENT SPECIFIC GRAVITY

The ratio between the weight of a unit volume of water-free soil and the weight of the same volume of water is spoken of as the apparent specific gravity or volume weight of a soil. It is obtained by dividing the weight of a certain volume of soil, in grams, by the volume occupied by that soil in cubic centimeters. No account is taken of the pore or air space.

Determination of Apparent Specific Gravity:

1. Fill a 2- by 12-inch tube or a 500-cubic centimeter graduated cylinder with dry soil. Weigh carefully. Repeat the process three times for each soil. Take the average of the three weighings.

2. Fill the tube with water to exactly the same level and weigh. If graduates are used, the volume in cubic centimeters of water will equal the weight in grams of the water.

3. Divide the weight of the soil by the weight or the volume of water, and designate the result as the apparent specific gravity.

4. Report on cards headed as follows: columns (1), (2) and (3), weight of tube and air-dry soil; (4) average weight; (5) weight of dry tube; (6) average weight of air-dry soil; (7) weight of volume of water corresponding to volume of soil; (8) apparent specific gravity.

Suggested Experiments

1. The weight of a cubic foot of water is 62.42 pounds. Determine the weight of a cubic foot of each soil.

2. An acre of soil covers an area of 43,560 square feet. Determine the weight of an acre foot of each soil type. Determine the weight of each soil to a depth of $6\frac{2}{3}$ inches.

3. The apparent specific gravity varies with the degree of compaction. Determine the apparent specific gravity after the soil has been well compacted, by dropping the tube several times on an open book from a height of 6 inches.

4. Determine the influence of moisture as affecting compaction by using soils with 10 to 20 per cent moisture content, making the proper deductions for moisture added.

5. Determine the hygroscopic moisture content and deduct it from the air-dry weight, reporting the apparent specific gravity of the water-free soil.

6. Determine the apparent specific gravity of field soils by forcing, a tube ($1\frac{1}{2}$ to 2 inches in diameter) into the ground to a certain depth 6, $6\frac{2}{3}$, 8, 10, or 12 inches. Remove the soil from the tube and place in a cloth sack or in a pan to be taken to the laboratory where it is first air-dried and then dried to remove the hygroscopic moisture. The same tube may be used to collect a number of samples. Determine the volume of the tube by filling to the same level with either water or clean sand and measuring the volume in cubic centimeters. Calculate, using columns 6, 7, and 8 above.

THE REAL SPECIFIC GRAVITY

The real specific gravity of the soil is the relationship of the volume weight of the solid particles of soil compared with that of

water. As it is not possible to get all the particles into a solid mass, the space between the particles is filled with water. Hence, it is necessary to free the soil particles of all traces of water, or better, to determine the hygroscopic moisture before determining the real specific gravity.

Determination of Real (Actual) Specific Gravity:

1. Fill a 50-cubic centimeter pycnometer with freshly boiled, distilled water that has been cooled to 20°C., and adjust capillary tube so that it is exactly full, wipe dry, and weigh on balance to 0.1 milligram.

2. Pour out about one-half of the water, wipe dry, and again weigh.

3. Add approximately 5 grams of soil or half as much muck or peat, and again weigh. The increase in weight is the weight of the soil added. This weight should be carefully noted.

4. Carefully boil the water containing the soil, in the pycnometer on an asbestos mat for a few minutes to expel air particles.

5. Allow to cool for a few minutes, then place in a pan of cold water and cool to 20°C., wash off the thermometer with a few drops of the same water, replace capillary tube after adjusting the water so that tube is exactly full, wipe dry, and again weigh.

6. The weight of the water-free soil divided by the weight of the water displaced equals the specific gravity.

7. Report on cards headed as follows: column (1) weight of pycnometer filled with water; (2) weight of pycnometer half-full of water; (3) weight of pycnometer half-full of water plus soil; (4) weight of soil added; (5) per cent of hygroscopic moisture in soil: (6) actual weight of dry soil; (7) weight of pycnometer filled with water and soil; (8) increase in weight due to addition of soil; (9) column (6) less column (8) equals the weight of water displaced; (10) specific gravity.

INTERNAL SURFACE AREA OF A SOIL

The internal surface area of a soil is the combined surface area of each of the soil particles.

Determination of the Internal Surface Area of a Soil:

1. Prepare soil fractions (Sec. 1) or separate the soil into different sizes as in a mechanical analysis.

2. Determine the number of particles in 0.01 gram of each of the water-free samples, by placing the weighed sample on a slide,

thoroughly mixing with a few drops of water, spreading evenly, and counting the number of particles under the microscope. The slide is accurately moved by means of a mechanical stage.

3. By means of the micrometer, measure a number of particles on each slide and average these measurements.

4. Assuming that all the particles are spherical, calculate the surface area by the use of the following formula: Area of a sphere $= 4\Pi R^2$ or ΠD^2.

Determination of the Volume of a Soil Particle:

The volume of a soil particle may be calculated from the data obtained in the determination of the internal surface area by the use of the following formula:

$$\text{Volume of a sphere} = \tfrac{4}{3}\Pi R^3 \text{ or } \tfrac{1}{6}\Pi D^3$$
$$\Pi = 3.1416.$$

SOIL MOISTURE

The relationship of moisture to the physical, chemical, and biological processes in the soil is of prime importance, especially from the standpoint of plant growth.

Expressions of Soil Moisture.—The amount of moisture present in field soils is usually expressed as per cent of dry weight of the soil, but may be expressed as pounds per cubic foot or as surface inches.

Determination of the Percentage of Moisture in a Soil:

1. Weigh out 100-gram portions of the moist soil in duplicate and allow to dry at room temperature for approximately 72 hours.

2. When thoroughly dry, weigh and calculate the per cent of moisture on the dry basis as follows:

Assume that the weight of the moist sample is 100 grams and that the weight of the air-dry sample is 78 grams. Then, the original soil sample consisted of 78 grams of soil and 22 grams of water. The 78 grams contained 22 grams or approximately 28.8 per cent of moisture.

Expressing Soil Moisture as Pounds per Cubic Foot. A cubic foot of soil varies in weight from 31 pounds in the case of peat to 106 pounds in the case of sand. The weight may be determined by multiplying the weight of an equal volume of water by the apparent specific gravity of the soil. By determining the per cent of moisture in the soil and determining the dry

weight of a cubic foot of that soil the pounds per cubic foot may be readily calculated.

Expressing Soil Moisture as Surface (Acre) Inches.—To express the moisture of the soil as surface inches, one merely determines the per cent of moisture and calculates it to pounds per acre for the desired depth. An acre-inch of water weighs approximately 226,000 pounds.

KINDS OF MOISTURE IN SOILS

There are three kinds of moisture in soils, hygroscopic, capillary, and gravitational.

Hygroscopic Water.—This is the soil water that cannot be evaporated under ordinary conditions. It is small in amount and is bound to the soil particle by physical forces. It varies in amount with the temperature and humidity of the atmosphere.

Capillary Water.—This is the soil water that may be evaporated at ordinary temperatures. It is governed more by the physical forces (adhesion and surface tension) than by gravitation and may move in any direction in the soil.

Gravitational Water.—This is the soil water that is governed at all times by the forces of gravity. The line between capillary and gravitational water is very broad and cannot be determined experimentally with any degree of accuracy.

Determination of Hygroscopic Moisture:

1. Reduce approximately 125 grams of air-dry soil to constant weight by exposing in a pan to the laboratory air for 3 days. Weigh at intervals.

2. Reduce two crucibles or weighing bottles to constant weight by heating at 110 to 120°C. for 30 minutes, placing in desiccator, cooling, and weighing.

3. Weigh out 50-gram portions of the air-dry soil and place in the tared crucibles or weighing bottles. Place in drying oven at 105 to 110°C. for 8 hours or more.

4. Remove from oven, place in desiccator, cool and weigh quickly. Repeat the process until the weights are constant. If crucibles are used, the material may later be used for the determination of organic matter by the "Loss on Ignition" method, page 40.

5. Calculate the per cent of hygroscopic moisture and record on card, using the following headings: column (1) Weight of crucible; (2) Weight of crucible-air-dry soil; (3) Weight of air-

dry soil; (4) Weight of crucible-soil after heating 8 hours; (5) Weight of water-free soil; (6) Loss in weight (hygroscopic moisture); (7) Per cent of hygroscopic moisture based on weight of water-free soil.

Suggested Experiments

1. Determine the hygroscopic moisture in different-textured soils as sand, loams, clays, peat, etc., in samples taken at different depths and in the soil fractions.

2. Determine the ability of the moisture-free soils to absorb moisture again by exposing the sample to the laboratory air and noting the increase in weight.

Determination of Capillary Moisture:

1. Secure a tube 1 to 2 inches in diameter and 12 to 15 inches long, place a piece of cheesecloth over the bottom, weigh and record the weight.

2. Fill the tube with air-dry soil to the same depth at which the sample was taken, 6, 8, 12 inches or deeper. (If no tubes are available, tall lamp chimneys may be used.) If moist soil is to be used the per cent of moisture must be determined.

3. Compact the soil by allowing the filled tube to drop a short distance several times; a drop of from 4 to 6 inches is usually sufficient. Record the weight of the filled tube.

4. Place the base of the tube in a pan of water, and allow the water to rise by capillarity for from 24 to 72 hours, or until the surface of the soil is moist.

5. When the water has reached the surface remove the tube, place on a blotting pad for a few minutes to remove any excess water, and again weigh.

6. Report findings on card headed as follows: column (1), Weight of empty tube; (2), Weight of filled tube; (3), Weight of dry soil in tube; (4), Weight of tube and wet soil; (5), Weight of water taken up by soil; (6), Weight of water in original sample; (7), Weight of capillary water in soil; (8), Per cent of capillary water; (9), Time required for surface to become moist.

7. Save the tube containing the soil for the determination of gravitational moisture.

Suggested Experiments

1. Determine the effects of adding various amounts of organic matter, *i.e.*, 5 to 10 per cent or more of peat or well-rotted manures, upon the rise of capillary moisture, noting the effect both on the time of rise and the amount of water.

2. Determine the effect of additions of small amounts of lime, 0.1 to 1.0 per cent, on the rise and amount of moisture. Substitute soluble salts for lime.

3. Calculate the capillary moisture in pounds per cubic foot, or in acre-inches.

4. Use glass tubes and note the height of rise from time to time.

5. Compare the effect of per cent of water in the soil by using an air-dry soil, compared with the same soil containing 4, 8, 12 or more per cent of moisture.

6. Prepare one set of tubes and place in the water at different angles, *i.e.*, inclined at 20-degree intervals from the vertical and note the relation between the angle and the rate of movement of the moisture.

7. Determine the capillary moisture of field soils by using samples secured in tubes as directed in (6) "Suggested Experiments" under the "Determination of Apparent Specific Gravity," page 24.

8. The distribution of moisture in Soil columns (United States Department of Agriculture, *Bulletin* 1221) may be determined by the use of $1\frac{1}{4}$- to $1\frac{1}{2}$-inch nickel-plated brass tubing, such as is used by plumbers. If the tubes are cut into 1-inch lengths and the ends milled, a fairly tight joint (not air-tight) may be secured. The bottom section should have a piece of fine-meshed, brass-wire gauze soldered over the lower end to retain the soil. The column may be built as high as desired. If desirable, the joints may be sealed by wrapping with friction tape. A trough, made by nailing two strips of wood together, is desirable in holding the sections rigid.

The movement of capillary moisture may be accurately determined in long-time experiments by the apparatus.

Determination of Gravitational Moisture:

1. Place the tube containing the moist soil, from the capillary determination, in a bucket of water with the level of the water the same as that of the soil and allow to remain until thoroughly saturated. Note the time required for saturation.

2. Remove, wipe dry, place tube in beaker to catch any excess drainage water and weigh.

3. Report on card, headed as follows: Column (1), Weight of tube + soil + capillary water; (2), Weight of tube + saturated soil + beaker; (3), Weight of beaker; (4), Weight of gravitational water; (5), Per cent of gravitational water; (6), Time required to saturate.

4. Calculate in pounds per cubic foot or in acre-inches.

6. Calculate the per cent of each of the three kinds of moisture in each of the different soils studied.

Suggested Experiments

1. Cover the tops of the tubes to prevent evaporation, and allow to drain, weighing at intervals, until the tubes are of the same weight as at the

beginning of the experiment. From this data note the influence of the various soil types upon the movement of the gravitational water.

2. Determine the influence of the various additions, as suggested under capillary moisture, upon the movement of gravitational water.

3. Determine the rate of percolation of water through the soil, in the tubes, by placing the tube in a clamp so that the lower end is several inches above the table top. Keep a layer of water on top of the soil in the tube; catch any surplus water in a beaker at the bottom. Determine the amount of water that will percolate through the soil in 30, 60, or 90 minutes.

Determination of the Optimum Moisture Content of Soil:

The optimum moisture content of a soil is that per cent of moisture which will best support plant growth. Usually the optimum is approximately 50 per cent of saturation, but in sandy soils it is higher, 50 to 65 per cent. It may be quickly determined as follows:

Place 100 grams of air-dry soil on a moist filter paper, in a funnel, supported in the neck of a Florence or Erlenmyer flask. Weigh the entire apparatus. Saturate the soil, allowing any excess to drain in the flask, empty the excess water, and again weigh. The increase in weight is the amount of moisture necessary to saturate the sample. If the soil is a loam 50 per cent of this amount may be assumed as the optimum.

RELATION OF SOIL MOISTURE TO SEED GERMINATION

1. Place 250-gram portions of air-dry soil in eight tumblers.

2. Adjust the moisture content as follows: (1) 5 per cent, (2) 10 per cent, (3) 15 per cent, (4) 20 per cent, (5) 25 per cent, (6) 30 per cent, (7) 35 per cent, and (8) 40 per cent.

3. Plant 10 to 20 grains of oats or wheat in each tumbler. Cover with watch-glasses to prevent evaporation and weigh, noting the total weight on the cover.

4. Examine every other day for 2 weeks. Weigh every third day and replace any water lost by evaporation.

5. Report on cards headed as follows: Column (1) Per cent of moisture in soil; (2) Weight of soil, tumbler, and cover; (3), (4) and (5) Amount of water added to replace that lost by evaporation; (6) Number of days required for germination; (7) Number of plants developed; (8) Total weight of green plants; (9) Relation of growth to optimum moisture content.

Determination of Factors Affecting Losses of Soil Moisture:

1. Fill five 250-cubic centimeter low-form beakers, to within $\frac{1}{2}$ inch of the top, with a loam soil, and thoroughly saturate with water.

2. Fill one beaker to the top with dry sand, another with dry loam, another with clay, another with wheat chaff and plant the last thickly with wheat or oats.

3. Place in the sunlight and weigh every day for 10 days.

4. Prepare a similar series and place where an electric fan may blow across the beakers for 8 hours each day.

5. Record results and explain fully.

Suggested Experiments

1. Determine the surface area of each of the above beakers, (area of a circle = ΠR^2) and calculate the evaporation from 1 acre. 1 acre = 43,560 square feet.

2. Special evaporimeters equipped with a water jacket may be purchased for the exact determination of the rate and amount of evaporation. Comparative results may be secured by the use of battery jars, deep crystallizing dishes, etc.

SOIL AIR

The amount of air in soil is equally as important to the physical, chemical, and biological properties of the soil as the amount of moisture. There must always be a ratio between the water and air content of the soil for plant growth.

POROSITY OF SOILS

Porosity is the total amount of air space in the soil and like moisture is usually expressed in per cent of volume. The porosity of each of the soils and the soil factions should be determined.

Determination of Porosity:

No equipment is necessary provided the data in the preceding experiments is available. Calculate the per cent of porosity from the apparent specific gravity and real specific gravity determinations using either of the following formulas:

$$\text{Porosity} = \frac{\text{Real specific gravity} - \text{Apparent specific gravity}}{\text{Real specific gravity}}$$

$$\text{Porosity} = 100 - \left\{ \frac{\text{Apparent specific gravity}}{\text{Real specific gravity}} \times 100 \right.$$

$$\frac{(\text{Volume of soil} \times \text{Real specific gravity}) - \text{Weight of water-free soil}}{\text{Volume of soil} \times \text{Real specific gravity}} \times 100.$$

Determine the porosity of field soils and correlate with the tilth of those soils.

Determination of Rate of Air-flow through a Soil Column:

This determination requires a specially constructed tube, closed at the bottom, with a perforated false bottom, approximately 1 inch above the bottom, and a nipple suitable for attaching rubber tubing leading from the space between the false and true bottom. Fair results may be secured by the use of a percolation tube tightly stopped in a 500-cubic centimeter suction flask.

1. Fill the tube very carefully with the air-dry soil, avoiding compaction.

2. Connect the nipple, by means of pressure tubing, to a 5-liter aspirator bottle filled with water.

3. Allow the water to flow from the aspirator bottle and collect in a 1,000-cubic centimeter graduate.

4. Note the time required to draw through 1 liter of air through the soil.

5. Make similar observations on all types of soil, and the soil fractions. Note the influence of size of particles, of compacting and of wetting on the rate of flow.

Suggested Experiments

Determine the rate of flow of air in field soils at various depths by using a ½-inch brass tube drawn out to a sharp point and perforated with a number of small holes 1 or 2 inches from the point. Push the tube into the soil until the perforated portion is at the desired depth and draw air through by suction, as in the above. Two 2,500-cubic centimeter bottles may be used instead of the aspirator bottle and graduate. As a bottle becomes empty it is replaced by the filled one. A plane table or a small folding table may be used as a support for the bottles.

THE CARBON-DIOXIDE OF SOIL AIR

The above field apparatus may also be used for the determination of the carbon-dioxide of the soil air by placing an absorption tower containing 50 to 100 cubic centimeters of 0.4 per cent sodium hydroxide between the brass tube and the suction bottle. The sodium hydroxide will absorb the carbon-dioxide from the soil air as it bubbles through. The amount of carbon-dioxide absorbed may be determined as described under "Titration of Carbon-dioxide," Sec. 3, page 119.

Determination of Power of Capillary Moisture to Dispiace Soil Air:

1. Arrange a tube of soil as directed for capillary moisture.

2. Stopper tube tightly with a one-holed rubber stopper fitted with a glass tube.

3. Invert a 1,000-cubic centimeter graduated cylinder, filled with water, in a large pan or beaker and support by means of clamps so that the lower end of the cylinder is about 1 inch from the bottom of the pan.

4. Adjust a rubber tube, connected with the stopper in the soil tube, so that the free end is under the graduated cylinder.

5. Place the soil tube in a pan of water as in the determination of capillary moisture.

6. As the capillary water rises in the soil the air will be displaced, and it will in turn displace the water in the graduate.

7. Make observations from time to time noting the number of cubic centimeters of air displaced.

Suggested Experiments

1. Determine the influence of the different textured soils, the influence of organic matter, lime, etc., on soil-air displacement.

2. Determine the influence of moisture by comparing a dry soil with the same type of soil containing various amounts of water.

3. Determine the retarding influence of dry soil by filling the tube the first 4 inches with moist soil (8 per cent), then 2 to 4 inches with air-dry soil and then another layer of moist soil. Observe carefully the amounts of air displaced at 20-minute intervals.

SOIL TEMPERATURE

Soil temperatures are chiefly influenced by the texture, structure, organic matter, color, water, topography, vegetation, and methods of tillage.

Determination of Temperature of Field Soils:

Determine the temperature of sod, cultivated and compact soils to show the effect of the condition and covering of the surface on the temperature. Also determine the temperature of dark- and light-colored soils under each of the above conditions. Note the difference in temperature of south and north slopes in order to secure the effect of topography. Take the temperature readings to a depth of 4 to 6 inches, making the depths the same in all cases. Compare the soil temperature with that of the air taken 4 feet above the soil.

HEAT CONDUCTIVITY

Conductivity of heat is the molecular transmission of energy from one point to another. The rate of transmission depends upon composition, texture, structure, and moisture content of

the soil, as well as upon the length of time, distance, and intensity of the heat.

Determination of Heat Conductivity by Soils:

1. Place the soil in the tray of a specially constructed heat-conductivity apparatus.
2. Fill the copper vessel with water.
3. Insert thermometers of standardized accuracy, $2\frac{1}{2}$ to 3 inches deep in the soil, at 1-inch intervals from the copper vessel and record the temperatures.
4. Heat the water to 95°C. Record the temperature of each thermometer at 10-minute intervals, for 1 hour.

THE SPECIFIC HEAT OF SOIL

Specific heat is the ratio between the amount of heat necessary to raise a given weight of the substance 1 degree and the amount necessary to raise the temperature of the same weight of water 1 degree. The specific heat of dry soil is approximately 0.25, but becomes greatly modified by the addition of water. Special apparatus is required consisting of a calorimeter and a thermometer graduated in $\frac{1}{10}$°C., or better a Beckmann thermometer.

Determination of Specific Heat:

1. Remove the hygroscopic moisture from approximately 100 grams of soil, place in a stoppered flask, and keep in the constant temperature apparatus for 24 hours.
2. Weigh the calorimeter cup. Calculate the weight of those portions of the stirrer and thermometer which are immersed in the water and calculate the water value of all from the specific heat of the material of which they are composed.
3. Place in the cup enough water accurately weighed, or carefully measured and at the same temperature of the bath, to make the water value of the system and water added exactly 200 grams of water.
4. Place stirrer and thermometer in the calorimeter, close and read the temperature until it becomes constant.
5. Quickly weigh out 50 grams of water-free soil and add to the calorimeter. Do not keep open any longer than is absolutely necessary. Close, stir, and read the temperature at half-minute intervals until the temperature becomes constant.

6. Calculate the specific heat using the following formula:

$$\text{Specific heat} = \frac{m(O - t)}{M(T - O)}$$

M = weight of the water-free soil. T = Temperature of the soil.
t = temperature of water in calorimeter before soil is added.
O = Constant temperature of water and soil in calorimeter.
m = grams of water used plus water value of calorimeter, etc.

SOIL CATALYSIS

The catalytic power of a soil is the ability of that soil to aid a certain reaction without entering into the end product of the reaction.

Determination of the Catalytic Power of Soils:

1. Prepare the following apparatus.[4] Fit three or four large, heavy-walled, bacteriological test tubes, with two-holed stoppers. In one of the holes place a short piece of glass tubing closed at one end with a rubber tube and a clamp. In the other, place a bent tube and attach a piece of rubber tubing 8 to 10 inches long. Fill a 50-cubic centimeter gas burette with water and invert in a 250-cubic centimeter beaker, half-filled with water, supporting the burette with a clamp. An ordinary burette may be used in place of the gas burette, if the height of the water column is adjusted at the 50-cubic centimeter point. Adjust a short bent tube in the free end of the long rubber tube and arrange so that any gas generated by the soil will displace the water in the burette.

2. Place 5 grams of each soil sample in individual tubes and connect in turn with the apparatus.

3. Add dilute sodium hydroxide to hydrogen peroxide until it is neutral or slightly alkaline to phenolphthalein. Dilute the solution until it has a strength of 1.5 per cent hydrogen peroxide.

4. Add 10 cubic centimeters of the 1.5 per cent hydrogen peroxide solution to the soil through the short tube, close clamp, shake at regular intervals and record the volume of oxygen evolved at intervals for 1 hour.

Suggested Experiments

1. Determining the effect of surface area by using the soil fractions and calculate the relation of surface area to the catalytic power.

2. Determine the effect of sterilization by comparing the catalytic power of fresh samples with those partially and completely sterilized (see "Soil Sterilization," Sec. 4, page 188).

SOIL TENACITY

The tenacity of a soil is the cohesive property which it possesses and is measured by the force necessary to break a column of soil of known diameter, usually 1 square inch. The force required varies with the amount of moisture present and the character of the soil.

Determination of Soil Tenacity:

1. Weigh out 250 grams of each sample and place in a large soil pan. Add 10 cubic centimeters of water and mix thoroughly with a spatula. Transfer to the tenacity apparatus, which has cages clamped firmly together. Pack firmly with the spatula, scrape away the excess, leaving the soil level with the top.

2. Carefully release the clamp and pour sand slowly into the bag until the column breaks. Weigh the sand and bag to the nearest gram and record the weight. This weight is representative of the tenacity of a column of soil 1 inch square.

3. If the apparatus is of the sliding type, the amount of friction in each case must be taken into account. This is determined by pushing the soil back from the contact edges and determining the weight necessary to pull the cages apart. This weight must be subtracted from all determinations.

THE MODULUS OF RUPTURE OF A SOIL

The modulus of rupture[1] is a determination of the amount of force required to break a soil briquette of known size and length. In other words, it is a method for determining the tenacity of a soil.

Determination of the Modulus of Rupture:

The Apparatus.—The apparatus required consists of a brass mold for making the briquette and a testing machine for measuring the amount of force required to break it.

The Mold.—The mold, illustrated in Fig. 1, is a rectangular box, made of brass, with a removable bottom. The inside dimensions are $11 \times 2.5 \times 1.95$ centimeters. The walls are about 1 centimeter thick and the bottom about $\frac{1}{2}$ centimeter. The size of the mold is merely suggestive.

The Testing Apparatus.—The testing apparatus, illustrated in Fig. 2, is a machine that is mechanical in operation. It consists of a balanced lever resting on the support A. The system of fulcra in relation to the application of the load are F_1, F_2 and F_3, the distance F_2 to F_3 is $\frac{7}{8}$ of the distance F_2 to a point directly over the point P_1. The system of fulcra responsible for the support of the briquette and the direct transmission of the load to it, during the test, are P_1, P_2 and P_3. With the whole system balanced upon F_2 by the addition of sand to, or the removal of

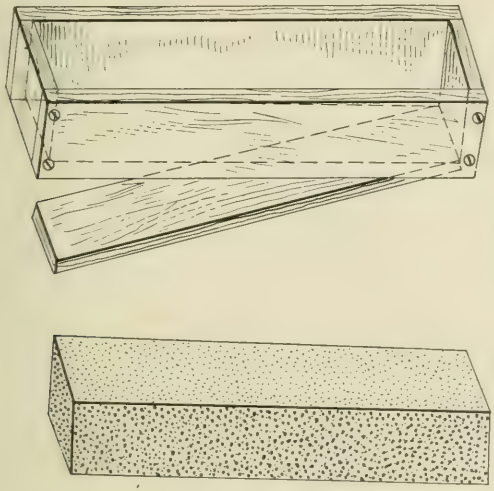

FIG. 1.—Briquette mold.

sand from, the vessel V_1 and with the briquette in place, water is released through the supply tube, to the vessel V_2, by dropping the lever at S. Before doing this, the lever C should be drawn back until the shaft R is so held, trigger fashion, by the spring D so that the tongue T (which later will be thrown out by the shaft R upon release of D) may spring in and hold down the bar M, which is directly responsible for the control of the water supply to V_2. The flow of water into V_2 develops a maximum load upon the briquette at P_1. At the time of rupture the system drops to the supporting arm K. This movement is regulated so as to cause the trip E, firmly attached to the supporting wire, to carry down the lever L. When L moves down it pulls down the spring D, releasing R, which shoots forward and throws out

the tongue T, thus permitting the control M to rise, by means of the spring B and so shut off the flow of water.

Making the Briquette.—Bring the moist field soil to a condition of maximum plasticity, as judged by pressing between

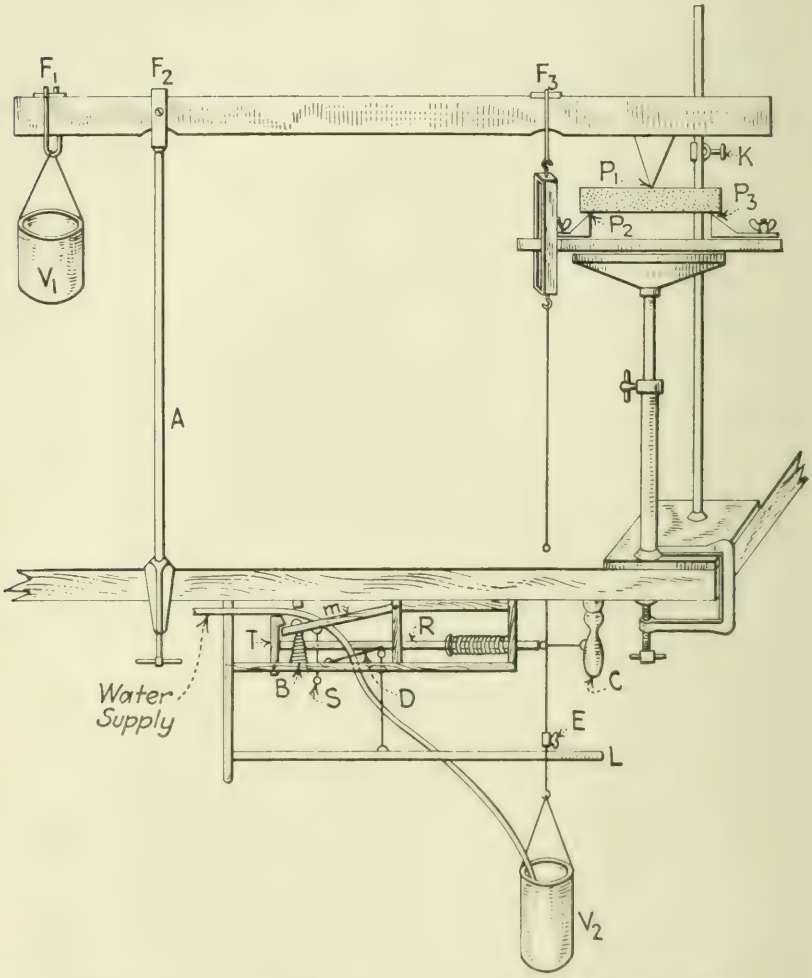

FIG. 2.—Apparatus for the determination of the modulus rupture.

thumb and finger. Coat the inside and bottom of the mold with paraffine oil. Press the moistened sample firmly into the mold and smooth off with a spatula. Invert the mold on a glass plate and carefully press out the briquette. Allow the briquette to

become thoroughly air-dry, turning several times to avoid checking. Dry in the oven at 105° to 110°C. overnight.

Making the Test.—Connect the various parts of the apparatus, making sure that the container V_2 is empty. Place the briquette on P_2 and P_3. Allow water to flow slowly, about 2 centimeters per second, into V_2. When the briquette breaks, measure or weigh the amount of water required.

Calculations.—The modulus of rupture is obtained by the application of the following formula:

$$R = \frac{3Wl}{2Bd^2}$$

R = Modulus of rupture.
W = Actual load in grams.
l = Length in centimeters between the supporting fulcra P_2 and P_3.
B = Breadth of the piece in centimeters.
d = Depth of the piece in centimeters.

The values found in this manner and expressed as gram-centimeters, represent the load that is required to break a piece of such material, under standard conditions, having a cross-section of 1 square centimeter.

Suggested Experiments

1. Determine the tenacity of soils of varying moisture content by using samples containing 2, 5, 7½, 10 per cent, etc., up to saturation. Tabulate the results and plot a curve for each soil, using the per cent of moisture as the abscissa and the modulus of rupture as the ordinate.

2. Determine the influence of organic matter by adding various amounts of muck, or the influence of lime, by adding small amounts of lime water and allowing to stand for 24 hours.

3. Determine the effect of size of particle or surface area upon tenacity, or modulus of rupture, by using the soil fractions prepared in Sec. 1.

4. Determine the effect of extreme heat on tenacity, or modulus of rupture, by igniting the soil and determining the tenacity of the ignited sample.

5. Fill several shrinkage pans with soil as described below. Cut the soil into strips so that it will form a block 1 inch square and 3 inches long. Allow to dry. Attach two short pieces of wood to the table so that they will project several inches over the edge and will be $2\frac{1}{2}$ inches apart. Place the dried strips of soil on the pieces of wood. Suspend a bag from the center of the soil strips. Pour in sufficient sand to break the strip and record the weight required. The size and length of the soil mass may be varied.

SHRINKAGE OF SOIL

When the moisture is removed from soils they will usually shrink. The amount of shrinkage is easily measured.

Determination of Shrinkage in Soils:

1. Weigh out a sufficient amount of soil to fill a pan 3 inches square and 1 inch deep. Place the soil in a mixing pan and add sufficient water to bring about maximum tenacity.

2. Place cheesecloth in the bottom of the pan, pack in the soil, scrape off even with the top, take out the block of soil on the cheesecloth and dry at room temperature for 48 hours. Measure carefully the size of the dry block.

3. Place the block in the oven at 100 to 105°C. for 24 hours and again measure.

4 Calculate the shrinkage and express in per cent of the original area of the block of wet soil.

ORGANIC MATTER OF SOILS

The organic matter content of a soil has an important bearing upon its physical phenomena. It may be roughly determined by simply igniting the sample.

THE LOSSES OF A SOIL WHEN IGNITED

The losses that a soil suffers when it is ignited are often taken as a measure of the organic matter content of that soil and prove the simplest method for its approximation. When ignited, the organic matter, volatile salts, and water of hydration will be driven off. In heavy clay soils and all fine grained soils, the water forms the greater portion of the loss. Consequently subsoils may lose as much weight as surface soils, due to the water content. The greater the amount of organic matter in a soil, the nearer the loss on ignition will correspond to the real amount. A peat soil for instance, may give a close approximation of the real amount of organic matter present.

Determination of Loss on Ignition:

1. Clean several small crucibles and mark them permanently, by placing several crystals of ferric chloride in a few cubic centimeters of ink, marking with a pen. Dry the ink and ignite until the black color of the ink has been replaced with a deep iron-rust

color. The mark is permanent when it cannot be removed by thoroughly scrubbing with any common cleaning compound.

2. Reduce the crucibles to constant weight.

3. Weigh out 5 grams of moisture-free soil. Air-dry soil may be used provided its hygroscopic moisture content is known. Place the soil in the crucibles and record the amount and kind of soil placed in each.

4. Ignite the soil at a cherry red for 15 minutes, or until the black color disappears, then the soil is cooled, stirred, and reheated.

5. Place in desiccator, cool and weigh. Reheat and obtain a constant weight.

6. The loss on ignition is the difference between the weight of water-free soil and the weight of the soil after igniting.

7. Report on cards headed as follows: Column (1) weight of crucible and air-dry soil; (2) weight of air-dry soil; (3) weight of crucible and water-free soil; (4) per cent of hygroscopic moisture; (5) weight of water-free soil; (6) weight of soil after ignition; (7) loss in grams due to ignition; (8) per cent loss on ignition.

SOIL HUMUS

Humus is a stage in the decomposition of organic matter and is primarily the result of biological action. Its presence usually has a marked influence on the physical, chemical, and biological properties of soils.

Determination of Humus:

1. Fit a Gooch crucible with an asbestos pad, (a disc of filter paper exactly fitting the bottom of the crucible may be used instead of asbestos).

2. Weigh out 10 grams of air-dry soil and place in the prepared crucible.

3. Attach the crucible to a suction flask and, using gentle suction, wash with successive portions of 1 per cent hydrochloric acid until all calcium is removed (see "Soil Adsorption," page 44, for test for calcium). When free of calcium remove the excess acid by washing with distilled water.

4. Wash the contents of the crucible into a 500-cubic centimeter stoppered cylinder or shaking bottle, using 500 cubic centimeters of 4 per cent ammonium hydroxide and allow to stand

with occasional shaking for 24 hours. Let stand at least 12 more hours to permit settling.

5. Filter the supernatant solution and evaporate an aliquot of 100 cubic centimeters to dryness, in a tared crucible, on the steam bath. Dry at a temperature of 105 to 110°C. to constant weight.

6. Ignite the residue and again weigh. Calculate the amount of humus in the sample from the difference in weight between the dried and ignited residues.

SOIL ADSORPTION

"Adsorption[3] may be considered as a concentration of a dissolved or dispersed substance upon the solid, liquid, or gaseous adsorbing surface. After concentrating there the adsorbed substance may react, polymerize, dissolve, be coagulated, or may crystallize slowly."

Every solid surface exhibits a certain degree of adsorption phenomena. The greater the surface area, or the finer the particles, the greater the adsorption effect. Every soil exhibits the property of adsorption. The degree may be more or less marked.

Experiments to Demonstrate Adsorption

Adsorption of a Base.—Shake a dilute solution of potassium chloride with cotton or with carbon black. Allow to stand 10 to 20 minutes, filter or decant and test both the original and filtered solution with indicators.

Adsorption of an Indicator.—Shake finely powdered orthoclase with water to which has been added a little phenolphthalein. No pink color develops. Allow to settle completely, decant, and add more phenolphthalein to the clear solution. A distinct color appears which may be decolorized by the addition of more powdered rock.

Adsorption by Humus.[3]—Mix a little humus and a weak solution of oxalic acid in a beaker. The humus will absorb the oxalic acid. Allow to settle. When the supernatant solution is clear suspend a collodion sack filled with water and calcium carbonate and attached to a glass tube, in the clear solution but not touching the layer of settled-out humus. The oxalic acid will diffuse into the sack and react with the carbonate to form the insoluble

calcium oxalate, thus removing practically all the adsorbed oxalic acid from the humus.

An extraction of the humus with hot water will fail to show any oxalic acid. Ignition of the humus shows that it is free of bases.

SOLUTIONS FOR THE DETERMINATION OF SOIL ADSORPTION

Make dilute solutions of each of the following by dissolving 1 gram of the salt in 1,000 cubic centimeters of distilled water. Place in separate containers and label clearly.

1. Nitrogen carriers—potassium or calcium nitrate and ammonium sulfate.
2. Phosphorus carriers—mono-potassium phosphate or mono-calcium phosphate.
3. Potassium carriers—potassium chloride or potassium sulphate.

Determination of Soil Adsorption (qualitative):

1. Place 100 cubic centimeters of each of the above solutions (excepting ammonium sulphate) in 250-cubic centimeter Erlenmyer flasks.
2. Add 50 grams of slightly moist or air-dry soil, shake thoroughly, and allow to stand 20 to 30 minutes.
3. Filter through a moist filter, pouring back the first portion of the filtrate in order to have the solution as clear as possible.
4. Place 10-cubic centimeter portions of the soil solution in test tubes. At the same time place the same amount of the original salt solution in duplicate tubes. Test for the presence of acids and bases in both solutions as follows, comparing qualitatively the amount of each.

Nitrates: Add a few drops of ferrous sulphate solution, shake, and allow concentrated sulfuric acid to run down the sides of the tube so that it will form a layer under the solution. Note appearance of the brown ring.

Chlorides: Use 1 cubic centimeter of the solution, add a drop of nitric acid and 1 to 2 cubic centimeters of 5 per cent silver nitrate solution. Note formation of white precipitate.

Sulfates: Add two to five drops of hydrochloric acid and 5 cubic centimeters of 10 per cent barium chloride solution. Note formation of white precipitate.

Phosphates: Add a few drops of ammonia, a piece of litmus, make neutral with dilute nitric acid, and add 1 drop in excess. Warm slightly and add 5 cubic centimeters ammonium molybdate solution. Note formation of yellow precipitate.

Potassium: Add a drop of acetic acid and 5 cubic centimeters of a 5 per cent solution of sodium cobaltic nitrite. Note formation of yellow precipitate.

Calcium: Warm slightly, add a little ammonia and filter off any iron that may be precipitated. To the filtrate, add a few drops of a saturated ammonium oxalate solution. Note the formation of a white precipitate.

Adsorption of Ammonium Sulfate.—1. Place 1 cubic centimeter of ammonium sulfate solution in 250-cubic centimeter Erlenmyer flasks, add 100 cubic centimeters of water and 50 grams of soil. Allow to stand and filter as above.

2. Place filtrate in a 50-cubic centimeter graduate. In another graduate place 1 cubic centimeter of the salt solution and build up to the 50-cubic centimeter mark with distilled water.

3. Add 5 cubic centimeters Nessler's solution (see Ammonia, Nesslerization, Sec. 3, page 100) to each graduate and compare the depth of the colors produced.

4. Test for the adsorption of sulfates.

Suggested Experiments

1. Determine the flocculating effect of lime by adding 0.1 gram of calcium oxide to 100 cubic centimeters of distilled water containing 2 grams of fine clay soil in suspension. Shake at 5-minute intervals for 1 hour. Examine a loopful of the suspension under the microscope and compare with a similar suspension without lime. By varying the amount of lime and centrifuging the suspension for short intervals, noting the time required for clearing, a satisfactory lime curve may be secured.

2. Determine the influence of lime upon adsorption by adding varying amounts of lime water to the soil. The amount to be added will depend upon the acidity of the soil (see Acidity, Sec. 3, "Procedure for Student Determination of Lime Requirement," page 91). If the amount added is not sufficient to thoroughly moisten the soil, add distilled water to saturation. Mix thoroughly and allow to become air dry. Determine adsorption, especially of phosphates, ammonia, and potassium upon the air-dry soil.

3. Ignite weighed quantities of the air-dry soil in evaporating dishes to a constant weight. Place in desiccators containing ammonium carbonate for 24 to 48 hours and again weigh. Drive off the adsorbed ammonia by ignition. The desiccator may be filled with ammonium hydroxide in which case the adsorbed hydroscopic moisture must be driven off before the adsorbed ammonia can be determined.

ADSORPTION BY SOIL COLLOIDS

In Sec. 1, soil colloids were defined as those particles remaining in suspension under all conditions of force. Such a definition gives no idea of the size of the particle. To overcome this difficulty, the United States Bureau of Soils assumes that all inorganic materials may be classified as collodial which can "be dispersed into particles less than 1 micron in diameter without subjecting the soil to a drastic chemical or physical treatment that would disintegrate the mineral particles." Thus, soil particles (soil colloids) may be separated and estimated by the Subsidence Method (Sec. 1, page 16). As these small particles have marked adsorptive properties, it is further assumed that the amount of colloidal material in the soil may be estimated by the application of the ratio:

$$\frac{\text{Adsorption per gram of soil}}{\text{Adsorption per gram of colloid}} \times 100 = \text{Per cent of colloid.}$$

Estimation of Soil Colloids by Adsorption.[1]—1. Measure the size of the clay particles obtained in the soil fractions. If they exceed 1 micron in size, bring the mass into suspension with an excess of water and pass through the separator. The speed of the separator should be so adjusted, that all particles larger than 1 micron will be separated out. Clean the separator and again pass the suspension through, running the machine at full speed.

2. Dry the parts and scrape off the precipitated soil particles. Place the precipitate in labeled containers. No attempt is made to determine accurately the amount of colloidal material obtained.

3. Determine the adsorptive value of the colloidal material and of the soil by one or all of the following methods and apply the above ratio.

Adsorption of Malachite Green.[2]—Shake 0.25 gram of colloid, or 1 gram of soil in an end-over-end shaker with 25 cubic centimeters of water and let stand overnight. Add enough $\frac{N}{10}$ sodium oxalate to precipitate any soluble calcium and shake 15 minutes. Now add a 0.2 per cent solution of malachite green oxalate, with repeated shaking, until the depth of color in the supernatant liquid is about the same as that of a check solution containing 0.0004 gram of dye per cubic centimeter. Record the amount of

[1] It is suggested that the student become acquainted with the methods presented in Sec. 3 before attempting the following determinations.

dye solution added. Insure complete adsorption by shaking 1 hour. Add 5 cubic centimeters of normal sodium chloride to coagulate the colloid, centrifuge and read the supernatant solution colorimetrically, against the check solution. (For making colorimetric readings, see "Soil Nitrates," Sec. 3, page 134.) Calculate the amount of dye adsorbed by subtracting the amount left in the supernatant solution from the amount added.

Adsorption of Ammonia.[2]—Dry 2 to 5 grams of colloid, or 5 to 10 grams of soil, for 18 hours at 110°C. Weigh quickly and place in U tubes. The U tubes should have side arms so that ammonia gas may be drawn over the dried material. Immerse the tube in boiling water and evacuate as completely as possible for 15 minutes. Replace the boiling water with an ice-pack. Allow ammonia gas to pass over the material in the tube for 2 to 4 hours. The gas should first pass through a train of soda-lime driers and the pressure should be adjusted, by means of a mercury manometer, to 1 centimeter above that of the atmosphere. When the mercury level, in the manometer, remains without perceptible change for 30 minutes, with the ammonia supply cut off and the exit end closed, the adsorption may be regarded as complete. The temperature of the ice-pack should be kept at 0°C. throughout the period of adsorption.

Determine the amount of ammonia adsorbed as follows: Disconnect the ammonia supply but do not disconnect the soda-lime train. Remove the excess ammonia by connecting to the filter pump and reducing the pressure to about 10 centimeters in the manometer. Release the pressure by allowing air to flow through the drying train. Arrange two bottles as absorption members of an aeration unit (see Aeration Unit, Sec. 3, page 53), and partially fill with a measured amount of a saturated boric acid solution. Connect the bottles between the filter pump and the U tube. Again reduce the pressure. Remove the ice-pack and replace with boiling water. Allow air to flow slowly through the apparatus for 30 to 40 minutes, until all ammonia has been displaced. Determine the amount of ammonia absorbed in the boric acid solution by titration, using methyl orange as indicator. If desired, the preliminary evacuation of ammonia in the apparatus may be dispensed with, and the results corrected by running a blank.

Adsorption of Water Vapor.[2]—Place 2 grams of colloid or 2 to 4 grams of soil, previously air dried and passed through a 100-

mesh sieve, in shallow weighing bottles. Place the uncovered bottles in a desiccator containing a large amount of 3.3 per cent sulfuric acid and evacuate the desiccator to 50 millimeters of mercury. Keep the desiccator to a temperature of 30°C. for 5 days. Weigh the moist samples, dry at 110°C. for 18 hours and again weigh. Correct the moist weights for any moisture on the bottles. The difference between the corrected moist weight and the dry weight represents the amount of water adsorbed by the sample.

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SECTION 3

SOIL FERTILITY

A soil is fertile when it is in such a state that all the factors affecting plant growth contained within it bear such a relationship to each other as to produce a favorable condition for its maximum productive capacity. The condition may be for a specific crop or it may be for several crops.

Soil fertility is a study of those factors affecting the productive capacity of a soil. The study recognizes the physical, chemical and biological properties of the soil and cannot be restricted to any one factor,[28] for instance humus, nitrogen, texture, etc.

LABORATORY EQUIPMENT

The student is now entering a field in which he is more or less independent of the watchful care of the instructor, furthermore he will be working with compounds as they occur in nature, consequently, there may be substances that will interfere with the accuracy of the determination in question. The ordinary gravimetric methods as used in quantitative and qualitative analysis do not suffice for all determinations, and a different method (volumetric analysis) must be used. The greatest accuracy, both in the determination itself and in the equipment, must be observed to avoid any question of the results.

The Balance.—It is important to remember that the accuracy of all chemical determinations depends upon the accuracy of the balance. Every laboratory should be equipped with at least one high-grade balance and one set of standardized weights. The greatest of care must be observed to protect both the balance and the weights from any laboratory fumes, corrosive substances, finger marks, etc., that may possibly change their accuracy. This balance need not be in continuous use. It should not be used promiscuously by students, but should be kept as a check upon the accuracy of the other balances in everyday use.

The Use and Care of Platinum.—Platinum is not oxidized in the air at any temperature, neither is it attacked by any single

acid. There are, however, many substances that attack and combine with it at low temperatures.

Platinum is attacked by solutions containing free chlorine, bromine, iodine, and ferric chloride, and when heated, by the caustic alkalies, the alkaline earths, nitrates, cyanides, the hydrates of barium and lithium, and compounds containing phosphorus or arsenic and the easily reducible metals, such as lead. It is also affected by the presence of organic matter, carbon. If gas is used, great care must be exercised to have the flame free of carbon, as the deposition of carbon on the platinum will blister it. Also organic matter containing phosphorus or silicon makes the platinum brittle and liable to fracture. The hot metal should never be handled with brass or iron tongs. Special platinum-tipped tongs, or those with special composition tips, are made for this purpose and should always be used. In other words, platinum should be used only in those cases where specifically recommended.

Hot crucibles should not be plunged into cold water to loosen their contents, and should not be rolled between the fingers. If possible each series of crucibles should be provided with a hard, wooden form, and the crucible shaped to this form after use.

The crucibles may be cleaned by rubbing with moist, round-grained, sea sand.

GLASSWARE

The laboratory should be equipped with a good grade of glassware. Tests have shown that the American makes are equal, if not superior, to any of the European makes. Pyrex has been found (Bureau of Standards, *Technologic Paper* 107) far superior to any of the wares tested. However, all of the American makes are good.

The glassware should be thoroughly cleaned, and in case of hydrogen-ion concentration determination equipment, should be soaked in a cleaning compound, to remove all soluble substances. In addition to the regular cleaning materials, bon ami, sapolio, etc., the following cleaning solutions will be found helpful in removing the various contaminating substances:

Soda Solution.—Boil the glassware in 5 per cent common washing soda. Wash in hot soap suds and rinse in distilled water.

Dichromate Cleaning Solution.—To remove soluble substanes from the glass itself; to remove organic matter, or to clean the

interior of burettes, etc., immerse the material or fill the apparatus with the following solution:

Potassium or sodium dichromate	80 grams
Water	300 cubic centimeters
Sulfuric acid (commercial)	460 cubic centimeters

Dissolve the dichromate in warm water, cool, add the sulfuric acid slowly. If properly prepared, the solution will be filled with small crystals. The material may be used repeatedly and will digest organic matter as long as the crystals are present. When the crystals disappear, the solution may still be used as a solvent for contaminating compounds in glass.

Alcoholic Potash.—Equal parts of concentrated potassium or sodium hydroxide and 95 per cent alcohol. This solution is useful in removing grease marks or in cleaning balsam from bacteriological slides.

Acid Alcohol.—Five per cent hydrochloric acid in 95 per cent alcohol. Useful in removing bacteriological stains from glassware.

Xylol.—A very useful solvent for wiping grease marks from glassware and especially valuable for removing grease pencil marks. A small piece of cotton is dampened with the xylol, the pencil mark wiped off, and the place wiped with a clean cloth.

The inside of burettes that have clumps of cock grease deposited on the sides may be cleaned by repeated washings with xylol, then with alcohol, and finally soaked overnight in the chromic acid cleaning solution.

Standardization of Glassware.[26]—Volumetric analysis depends, for its accuracy, upon the use of various forms of apparatus, which will contain, or deliver, a definite quantity of solution. The apparatus includes various-shaped vessels, cylinders, flasks, pipettes, burettes, etc., that are marked to deliver or to contain a certain amount of solution. In case the bore of the apparatus is of the same size throughout its length, it may be graduated, so that fractions of each measurement may be secured. The accuracy of fractional measurements will depend upon the uniformity of the bore, thus graduated cylinders only roughly measure the solution, while graduated burettes, with a much smaller bore, have a greater degree of accuracy. Cylinders, burettes, and pipettes are usually calibrated to deliver a certain amount of solution, while flasks are usually graduated to contain

or to deliver a certain amount of solution. The lower mark on the neck of a flask indicates the amount of solution that may be contained in that flask, while the upper mark indicates the amount that it will deliver. The difference is the amount of liquid that adheres to the inner surface of the flask.

As the bore of all glass tubes may vary in size, it is necessary that the capacity of all graduated apparatus be carefully tested. A series of flasks, burettes, and pipettes, graduated to meet requirements of the United States Bureau of Standards, may be used as a basis for calibrating the apparatus that is in general use, or it may be calibrated by the use of the Morse Calibrating Apparatus. A very common method is to standardize and calibrate the apparatus by weighing the solution, in the apparatus or as delivered. For general use, however, all the apparatus should be adjusted, or calibrated, among themselves, in order that their relations be accurately known. Thus the pipettes should deliver exactly the same amount as the burettes. For instance, the amount delivered by a 25-cubic centimeter burette should be equal to that delivered by the 25-cubic centimeter pipette and this amount should be one-tenth the amount contained in the 250-cubic centimeter volumetric flask, etc. As the measurements in such cases are relative, the temperature makes little difference. The unit of measurement should be fairly accurate.

Calibration by Weight.—A true cubic centimeter is the volume of 1 gram of water in vacuum at 4°C. A Mohr cubic centimeter, used as the unit of volume in volumetric work, is the volume of 1 gram of water, weighed in air, with brass weights at a temperature of 17.5°C. The Mohr cubic centimeter = 1.0023 cubic centimeters or a true liter of water weighed in air, would occupy a volume of 1002.3 centimeters at 17.5°C. The following table gives the weight in air of 1 true cubic centimeter of water, and the volume in true cubic centimeters corresponding to the weight in air of 1 cubic centimeter, or 1 gram of water at a range of temperatures usually found in the laboratory:

TABLE. 1.—INFLUENCE OF TEMPERATURE ON WEIGHT AND VOLUME OF WATER

Temperature in °C.	Weight in air of 1 true cubic centimeter of water, gram	Volume occupied by 1 gram of water, cubic centimeters
15	0.9981	1.0019
16	0.9979	1.0021
17	0.9977	1.0023
18	0.9976	1.0024
19	0.9974	1.0026
20	0.9972	1.0028
21	0.9970	1.0030
22	0.9967	1.0033
23	0.9965	1.0035
24	0.9963	1.0037
25	0.9960	1.0040
26	0.9958	1.0042
27	0.9955	1.0045
28	0.9952	1.0048
29	0.9949	1.0051
30	0.9946	1.0054

Calibration of Burette by Weight.—Clean burette thoroughly with cleaning solution, being sure that the water runs freely down the sides and does not collect in droplets. Boil about 500 cubic centimeters of distilled water and allow to stand until it acquires the temperature of the laboratory. Record the temperature of the laboratory. Fill the burette, being sure that it is free of all air bubbles, run out slowly (approximately 30 seconds) into an accurately tared beaker exactly 5 cubic centimeters, weigh and record the weight. Record accurately the weight of subsequent 5-cubic centimeter portions until the burette is empty. Refill the burette and check the results. Calculate the value in true cubic centimeters occupied by the different weights of water as follows: Assume that the temperature was 20°C. and that the weight of the first or any subsequent 5 cubic centimeters was 5.005 grams. From the table we know that the weight of 1 true cubic centimeter in air at 20°C. is 0.9970 gram, hence $5.005 \div 0.9972 = 5.02$ cubic centimeters.

The Preparation of Neutral, Carbon-dioxide-free Water.—There are many types of water stills on the market that will deliver a satisfactory grade of distilled water. There are times, however,

especially in the determination of acidity, or the standardization of solutions when a strictly neutral, carbon-dioxide-free water is desired. If water freed only of carbon dioxide is desired the same may be secured by aspirating the ordinary distilled water with carbon-dioxide-free air for approximately 4 hours. If exactly neutral conductivity water is desired, the distilled water is first made slightly acid with sodium acid sulfate $NaHSO_4$,[1] aspirated for 2 hours with carbon-dioxide-free air and then distilled, the first 500 to 1,000 cubic centimeters being discarded. The distilled water is caught in a container protected from carbon-dioxide contamination by suitable guards. The apparatus may be made continuous in operation by having the acidified water on a higher level than the boiling flask, conducting it into the flask by means of a siphon, the rate of flow being controlled by a pinchcock. The resultant water should be of such a degree of neutrality that 500 cubic centimeters will show a distinct brown on adding a few drops of alazarin red indicator and should react distinctly to the addition of 1 drop of $\frac{N}{50}$ acid or alkali. Some workers prefer to use a double continuous still, in which case the distillate from the first flask is conducted below the level of the solution in the second flask, the flames being so adjusted that the operation is continuous. The second flask contains a solution of barium hydroxide. Potassium permanganate may be substituted for the sodium acid sulfate in this case.

THE AERATION UNIT

The determination of ammonia and carbon may be best carried out by the use of the air current. The equipment, modified to meet the individual requirements of the particular determination, is simple and easily made. The principle of the aeration unit is that air is conducted, usually by means of a vacuum, by glass tubes through a series of solutions. The air enters through a tube that conducts it to the bottom of the solution, it rises to the top and displaces any gas that may have been formed, these gases are then conducted to the bottom of a container partially filled with a solution designed to absorb them. The unit requires

[1] Sodium acid sulfate: To a saturated sodium hydroxide solution, add drop by drop, from a burette, stirring constantly, concentrated sulfuric acid until the neutral point is reached, and then an excess of acid equivalent to the amount that was used in reaching the neutral point.

two flasks fitted with two-holed rubber stoppers, and four glass tubes. Two of these tubes are of such a length that they will reach to the bottom of the flasks and serve to expose the air to the action of the solutions in them. The other two tubes are just barely long enough to pass through the stoppers and serve to conduct the air away. The free ends of the tubes may be of any desired length and bent to any desired angle. The units may be connected in a series or train, in which case the exhausted air is used in the following unit, or they may be connected in parallel, each unit drawing its air supply from a common center.

Aeration Unit Connected in Series. Ammonia Determinations.—In the case of the aeration unit connected in series the air is drawn through the solutions rapidly enough to keep them thoroughly agitated. As used in the determination of ammonia, the unit consists of one 500- or 800-cubic centimeter Kjeldahl flask fitted with a two-holed, rubber stopper and one pint milk bottle, or wide-mouthed bottle of approximately the same capacity, also fitted with a two-holed stopper. The stopper fitting the Kjeldahl is equipped with two 4- or 5-millimeter tubes, one barely protruding through the bottom of the stopper, the other of such a length that it will reach as near as possible to the bottom of the flask at the exact point where it rests upon the table. As the flask will be inclined, the tube is bent about $2\frac{1}{2}$ inches from the lower end. The amount of bending is just enough to allow entrance of the tube into the neck of the flask. The bent end of the tube is also slightly belled with a carbon pencil to facilitate spreading the air. The free ends of both tubes are bent to such a degree that they will be parallel with the table when the apparatus is in position. The stopper, fitting the milk bottle, is also equipped with two tubes of the same size, bent at the free end to an angle of approximately 90 degrees, and of such length that they may be easily connected with the tubes from the Kjeldahls. These tubes are also of unequal length, the shorter will just reach through the stopper, the longer will extend almost to the bottom of the bottle. The end of the longer tube, instead of being belled, is carefully blown to make a thick-walled bulb, slightly larger than the tubing. This bulb, while hot, is punctured full of small holes with a hot platinum wire. The holes are made as small and numerous as possible in order that the bubbles passing through them will be small and the evolved ammonia will be easily absorbed by the acid solution. If it is not convenient to use

bulbs on the ends of these tubes, they may be fitted with short pieces of larger tubing and packed with cotton or glass wool. In this case the tube is pushed close to the bottom of the flask in order to prevent the packing being drawn out by the force of the vacuum. The length of the train will depend upon the ability of the pump to keep the mixture thoroughly agitated at all times. A guard is provided at the open end (where the air enters) to free the air of all gases that may later be absorbed. Also a mercury guard is provided between the pump and the unit next to the pump, to prevent any back suction in case of sudden stoppage. If a water pump is used it is also necessary to place a 500-cubic centimeter suction flask next to the pump to prevent any water being drawn back into the mercury.

Aeration Unit Connected in Parallel. Carbon-dioxide Determination.—In case the aeration unit is connected in parallel, the air is drawn very slowly through the solution, in order that complete absorption of the carbon dioxide may be secured. Each unit is supplied from a common center and discharges its air into a tube leading directly to the pump.

In the aeration method for the determination of carbon-dioxide, evolved by soils, the air is drawn directly from a large bottle equipped with sufficient tubes to reach each unit. The air entering this central supply bottle is first passed through concentrated sulfuric acid and then through two soda-lime tubes to remove all carbon dioxide. As carbon-dioxide experiments are run for long periods of time, and this dry air would soon affect the soils, the moisture of the air is restored by conducting the carbon-dioxide-free air through a dilute solution (15 per cent by weight) of sulfuric acid placed in the large central supply bottle.

The absorption flask is very important, as special precautions must be used to expose the evolved carbon-dioxide gas to the absorbing solution, in as small bubbles as possible, and for as long as possible. The gas may be conducted to the bottom of cylinders by a tube equipped with a bulb so adjusted that a portion of the solution will be continuously drawn up into the bulb. Another satisfactory method is the use of a tower filled with some material to break up the bubbles. In this case a 500-cubic centimeter suction flask, fitted with a large glass tube filled with beads, etc., may be substituted for the cylinder. The air is drawn in through the arm of the flask and out through the top of

the tube. This form of apparatus is efficient but difficult to wash.

In all cases the air is drawn from each of the units by a series of T tubes connected together and leading to the pump.[35, 11, 22 and 10]

Grinders.—There are many types of grinders, both hand and power, that may be used in preparing the sample. Green plants may be ground with a sausage grinder while the dried plant remains may be best ground with a spice mill. A disc pulverizer type of mill is very satisfactory for grinding large samples of soil. When the number of samples is small, or only a few each day are to be ground, the most satisfactory grinder for laboratory use is the bucking board and muller. This type of grinder has the advantage of being easily cleaned, has a low initial cost, and will grind all soils and rocks.

Marking the Apparatus.—Glassware may be marked by the use of the ordinary grease pencil. Bottles may be permanently marked by attaching an ordinary label, marked with India ink and coating it with melted paraffine, lacquer, or varnish. A satisfactory ink for marking glass may be made by dissolving 200 grains of shellac in methyl alcohol, straining through a muslin cloth, and adding slowly, with frequent shaking, a solution of ¾ ounce of borax dissolved in 6 ounces of water. The desired pigment is added to the borax solution. Crucibles may be permanently marked by placing a few crystals of ferric chloride in a few centimeters of common ink, and marking with a pen. Dry the ink and ignite until the black color of the ink has been replaced with a deep iron-rust color. The mark is permanent when it cannot be removed by thoroughly scrubbing with sapolio or bon ami.

Stopcock Grease.—The stopcocks on burettes may freeze unless they are well greased. A satisfactory grease may be made by dissolving over a low flame one part of rubber in one part of paraffine and two parts of vaseline. If pure gum rubber is available, use sixteen parts vaseline, eight parts pure rubber and one part paraffine. If not stiff enough add more paraffine.

Table Top Preparations.—Table tops may be made acid-proof and partially fire-proof by either of the following solutions:

Solution 1

4 parts copper sulfate
8 parts potassium permanganate
1 parts ferrous sulfate
100 parts water

250 grams copper sulfate
250 grams potassium chloride
2,000 cubic centimeters water

SOLUTION 2

18 parts of aniline oil	100 cubic centimeters aniline oil
12 parts hydrochloric acid	100 cubic centimeters hydrochloric acid
100 parts water	2,000 cubic centimeters water

Apply two or three coats of solution 1 to the surface of the table until a thick deposit of crystals is formed; rub off the excess crystals with a dry cloth and apply two or more coats of solution 2. When thoroughly dry apply two coats of linseed oil. When complete, the table top will be a deep permanent black.

Grinding Glass.—Stopcocks may be ground tightly by the use of the following:

45 cubic centimeters turpentine.
22.5 cubic centimeters ether.
31 grams camphor gum.

Wet the glass with the liquid and sprinkle with emery or carborundrum dust. Start the grinding with coarse dust and finish with fine dust. Turn the cock in one direction. Plane surfaces are ground on thick glass. For smoothing the edges, use a sheet of emery cloth moistened with the above solution.

SOLUTIONS

In the analysis of soils, a number of different solutions are required. Some of these may be roughly prepared and others are carefully standardized.

Standard Solutions.—Standard solutions are solutions in which the contents of a definite volume are approximately or accurately known, the accuracy of the preparation depending upon the use to which the solution is to be put. Roughly speaking, all solutions are standard, but are designated according to their content.

Per Cent Solutions.—A solution containing a certain per cent of some soluble substance. Thus a 5 per cent salt solution is merely 5 grams of salt dissolved in enough water to make the total volume 100 cubic centimeters.

Per Cent by Weight Solutions.—A definite weight of a substance is dissolved in enough water to make the total volume 100 cubic centimeters. Thus the above solution may be expressed as per cent by weight. Some compounds have a greater specific gravity than 1, hence a 5 per cent sulfuric acid solution by weight would contain 5 grams in 100 cubic centimeters of the total solution.

Saturated Solutions.—A solution made by saturating with the desired compound. This point is reached when no more of the compound will be dissolved at a certain temperature.

Per Cent by Volume Solutions.—A definite volume of a substance is diluted to a volume of 100 cubic centimeters. Thus a 5 per cent sulfuric acid solution, by volume, would be almost twice as strong as the same solution by weight. Usually a solution made by the use of liquids is expressed by volume.

Molar Solutions.—A solution in which a weight, in grams, of the compound equivalent to the molecular weight of the compound, is dissolved in distilled water and built up to exactly 1,000 cubic centimeters. Thus a molar magnesium sulfate solution would contain 120.42 grams of the compound in 1,000 cubic centimeters. Such a solution would be expressed as $\frac{M}{1}$ Mg SO_4. A weaker solution, *i.e.*, ½ molar, would be expressed as $\frac{M}{2}$. Molar solutions are usually made up as a basis for the feeding of plants.

Normal and Approximate Solutions

The determination of many soil and plant elements, depends upon certain reactions in solution. The limits of these activities must be accurately determined, hence the normal solution or its equivalent, the approximate solution, is most commonly used.

Normal Solutions.—A solution containing one combining weight of replaceable hydrogen, or its equivalent per 1,000 cubic centimeters.

A normal solution is expressed as N and the strength of the solution indicated by the figure used as a fraction, *i.e.*, $\frac{N}{2}$ represents half normal or one-half the strength of normal, or by the figure placed in front of the N, *i.e.*, $\frac{2N}{1}$ represents that the solution is two times or double the strength of normal.

Normal solutions are made from organic as well as inorganic compounds but they are usually made for a different purpose, which will be explained in the case of oxidizing and reducing solutions. Thus a normal oxalic acid solution would contain the gram equivalent of the molecule $\frac{(COOH)_2}{2}$.

Calculation of the Equivalent Factor of Elements in Normal Solutions.—As 1 cubic centimeter of any normal acid solution is exactly equal to 1 cubic centimeter of any normal alkali solution, the equivalent factor of any element or compound will be the same for all. Thus, 1 cubic centimeter $\frac{N}{10}$ ammonium hydroxide contains 0.003651 gram ammonium hydroxide of which 0.001401 gram is nitrogen, but 1 cubic centimeter of $\frac{N}{10}$ ammonium hydroxide or 1 cubic centimeter of any $\frac{N}{10}$ alkali is neutralized by 1 cubic centimeter of $\frac{N}{10}$ hydrochloric acid or any $\frac{N}{10}$ acid. Thus it follows that 1 cubic centimeter of any $\frac{N}{10}$ acid is equivalent to (or capable of neutralizing) 0.001401 gram of nitrogen, when that nitrogen has been converted into the form of ammonium hydroxide. For instance, in the Kjeldahl determination, the distillate may be caught in any $\frac{N}{10}$ acid and the excess titrated with any $\frac{N}{10}$ alkali and the difference multipled by the factor 0.001401 gram, will give the amount of nitrogen distilled or aerated over. The factor for other elements or for other strength of acids or alkalis may be determined in the same manner.

Approximate Solutions.—A solution made of approximate strength measured in terms of some other solution. Thus an approximate normal $\frac{N}{1}$ solution is one of such strength that it closely approximates in strength the solution to which it is compared. Such solutions are fully as accurate as normal solutions, provided the proper factor or relationship to the standard solution is determined, and are widely used when the number of determinations to be made do not warrant the time required to make up a normal solution, *i.e.*, for student laboratory use.

Calculating the Factor Relationship of Approximate and Normal Solutions.—Assuming that 20 cubic centimeters of approximately $\frac{N}{10}$ acid solution are found to be equivalent in strength to 25 cubic centimeters of a $\frac{N}{10}$ HCl solution, then 1

cubic centimeter is equivalent to 1.25 cubic centimeter of the $\frac{N}{10}$ acid, but 1 cubic centimeter of the $\frac{N}{10}$ acid is equivalent to 1.401 milligrams of nitrogen. Then 1 cubic centimeter of the approximately tenth normal solution is equivalent to $1.25 \times 1.401 = 1.75325$ milligrams. The factor 1.75325 is used then as the true value for nitrogen whenever this solution is used. The factor for each element and for each solution must be determined.

Standardization of Normal Solutions

Normal solutions are standardized by determining accurately the value or amount of concentration of the substance contained in them. The method of standardization to be used depends upon the chemical nature of the substance in solution. The following methods are recommended:

Gravimetric Methods.—(*a*) Determining the specific gravity of the solution either with a pycnometer, hydrometer, or Westphal balance. The concentration of the solution may be found by reference to the proper physical table (see Appendix, page 213). Useful for concentrated hydrochloric acid, ammonium hydroxide etc. A solution prepared in this manner is not strictly accurate, hence should be further standardized.

(*b*) Dissolving a weighed amount of a carefully purified substance in a definite volume. Certain salts [24,25] are now supplied for this purpose. Thus a $\frac{N}{10}$ oxalic acid solution may be made by weighing 6.3 grams of the pure salt $H_2C_2O_4 2H_2O$ and diluting to 1,000 cubic centimeters.

(*c*) Precipitating the acid radical from a measured amount of the solution to be standardized and weighing it as an insoluble salt. Used for hydrochloric and sulfuric acid solutions, see preparation of standard hydrochloric acid solution.

(*d*) Neutralizing a measured quantity of the solution and evaporating the whole to dryness in a tared vessel. This method is valuable in the standardization of sulfuric acid with ammonia, as an excess of the ammonia is readily driven off in the evaporation process. Thus 10 cubic centimeters of $\frac{N}{1}$ sulfuric acid should contain 0.49 gram of H_2SO_4 which is neutralized by the ammonia with the formation of ammonium sulfate. The amount

of ammonium sulfate that should be formed is calculated as follows:

$$H_2SO_4 : (NH_4)_2 SO_4 :: 98 : 132$$
$$49 : \quad x \quad :: 98 : 132$$
$$x = 0.66.$$

There should be 0.6600 grams of ammonium sulfate formed if the acid solution had been normal. From this figure the normalty of the solution may be determined.

Volumetric Methods:

(*a*) Titrating the solution against weighed quantities of a chemically pure substance (see (*b*) above).

(*b*) Titrating against a measured amount of another solution which has been accurately standardized by one of the above methods.

As it is usually easier to make and standardize an acid solution, rather than an alkaline solution, and then standardize the alkaline solution in terms of the acid, the preparation of the most common acid solutions only will be discussed. The principle of the method may be applied to any solution necessary to be prepared in the following determinations:

Preparation of a Half-normal Standard Hydrochloric Acid Solution.—1. Determine the exact specific gravity of the concentrated acid to be used.

2. Calculate the amount of hydrochloric acid in 100 cubic centimeters of the concentrated acid.

3. Calculate the amount of the strong acid that must be used to make the approximate normalty of the desired volume; 2 to 5 liters are usually prepared. The volume must be exact and the amount of acid used should be slightly in excess of the calculated amount.

Calculations.—Assume that the specific gravity of the concentrated acid is 1.120. By reference to the specific gravity table, we find that it contains 23.82 per cent HCl, by weight, *i.e.*, 100 cubic centimeters contains 23.82 grams. But a $\frac{N}{2}$ HCl solution should contain 18.229 grams in each 1,000 cubic centimeters. Hence.

$$23.82 : 100 :: 18.229 : x$$

$x = 76.5$ cubic centimeter of the strong acid required.

As the solution should be slightly stronger than the calculated normality, 78 to 80 cubic centimeters of the concentrated acid should be used and diluted to exactly 1,000 cubic centimeters.

Standardization of a Half-normal Hydrochloric Acid Solution

Standardization of the Solution.—With a previously standardized pipette, calibrated to drain for ½ minute and then to be touched to the side of the flask, draw off four 25-cubic centimeter portions and place in 250-cubic centimeter Erlenmyer flasks. Dilute each portion to approximately 75 cubic centimeters with neutral distilled water and add at once, from a burette, sufficient 5 per cent silver nitrate solution to precipitate nearly, but not quite, all the chlorine. Close the flask with a clean stopper and shake until the precipitate will settle almost completely in a short time. Add more silver nitrate, in 1-cubic centimeter portions, until the precipitation is complete, avoiding

$$HCl + AgNO_3 = AgCl + HNO_3$$

more than 1 cubic centimeter in excess. Shake thoroughly and wash by decantation 3 times, (shaking thoroughly each time) using 100 cubic centimeters of water to which has been added 1 cubic centimeter nitric acid, decanting the liquid through an ashless filter. Transfer the precipitate to a glazed paper and burn the filter paper in a tared porcelain crucible. Add 2 to 5 drops of concentrated nitric acid to dissolve the reduced silver and then add 2 to 5 drops of hydrochloric acid. Evaporate carefully to dryness, avoiding spattering; and add the main precipitate; dry to constant weight at 120–130°C.; cool in a desiccator and weigh.

Calculations of HCl in the Original Solution.—Assume that the weight of the silver chloride precipitate is 1.8775 grams. By proportion, we find the molecular weight of hydrochloric acid will be to the weight of hydrochloric acid in the solution, as the molecular weight of silver chloride will be to the weight of the silver chloride in the precipitate. Thus

$$36.458 : x :: 143.4 : 1.8775$$

$$x = 0.4773 \text{ weight of HCl in the solution.}$$

As 25 cubic centimeters contains 0.4773 gram of HCl, then 1 cubic centimeter would contain 0.019092 gram, or 1,000 cubic centi-

meters contains 19.092 grams. As 1,000 cubic centimeters of $\frac{N}{2}$ HCl should contain 18.229 grams of HCl, the solution is slightly stronger than is required.

Dilution of Approximately $\frac{N}{2}$ HCl to Exactly $\frac{N}{2}$.—The amount of neutral water to be added to the above solution to make it exactly $\frac{N}{2}$ is calculated as follows: Assume that exactly 5,000 cubic centimeters of the original solution have been made and that 100 cubic centimeters have been withdrawn for testing purposes. By proportion, we find that the HCl in an exactly $\frac{N}{2}$ solution is to the amount in the tested solution as the total amount, in cubic centimeters, of the tested solution is to the total amount of that solution were it exactly $\frac{N}{2}$. Thus.

$$18.229 : 19.092 :: 4{,}900 : x$$

$$x = 5{,}132.$$

As the total amount of the solution should be 5,132 cubic centimeters and now is 4,900 cubic centimeters the addition of 232 cubic centimeters of neutral water is required to make it exactly $\frac{N}{2}$.

Build up to the required volume and keep for the standardization of alkali solutions as needed. The pipette used in measuring off the 25-cubic centimeter portions should be kept and used to measure off from this standardized solution the amounts required in the standardization of other solutions. This procedure will avoid the standardization of the burettes used in connection with the other solutions.

Preparation of Hydroxide Solutions.—The preparation of hydroxide solutions varies according to the base used.

(*a*) **Ammonium Hydroxide.**—Determine the exact specific gravity of the ammonium hydroxide to be used, and from the specific gravity tables determine the number of grams NH_4OH per cubic centimeter of the concentrated solution. Calculate the amount in cubic centimeter to be diluted so that the concentration will be slightly stronger than the normalty desired, in the same manner as directed in the preparation of a $\frac{N}{2}$ HCl. (For standardization see page 64.

(*b*) **Sodium or Potassium Hydroxide.**—Calculate the amount of pure salts to be weighed out in order to make the desired normality. Weigh quickly and build up to the desired concentration. As it is almost impossible to secure these salts free from carbonates, a slight excess over the calculated amount should be used. Allow the solution to stand overnight, or until it has settled clear, siphon off the clear solution as quickly as possible to avoid contamination with atmospheric carbon-dioxide, or proceed as directed for carbon-dioxide-free alkali under hydrogen-ion concentration. Titrate 10 cubic centimeters in the cold, using phenolphthalein, against a standard acid. Repeat the titration in a boiling solution. If there is a difference in the titrations, the presence of carbonates is assured. These carbonates may be removed by precipitation on the addition of a small amount of 10 per cent barium chloride solution. The latter procedure is usually not necessary, if proper care has been used in the preparation of the solution.

(*c*) **Calcium Hydroxide.**—Add 5 grams of pulverized calcium oxide to 2,000 cubic centimeters of carbon-dioxide-free water. Keep tightly stoppered and shake several times a day for 2 days, then allow to stand quietly for 1 week. As the resulting calcium hydroxide solution has a great affinity for atmospheric carbon-dioxide, special precautions must be taken to prevent contamination by using CO_2 guards in every instance where the solution may come in contact with the air. The solubility of $Ca(OH)_2$ is so slight that only the weaker solutions are made, for instance $\frac{N}{28}$.

Standardization of a $\frac{N}{2}$ Hydroxide Solution

All of the above hydroxide solutions may be standardized by the following process, adjusting the calculations to the normality desired, and making a little stronger than necessary in order that they may be properly diluted during the process of standardization.

Select a 25- or 50-cubic centimeter burette graduated in $\frac{1}{10}$ cubic centimeter, which is to be used in measuring the same solution throughout the balance of the laboratory work, and clean thoroughly. As the stopcocks of hydroxide burettes are liable to freeze, especially if not used regularly, it may be advisable to select a burette provided with a rubber tube and pinchcock.

With the pipette used in standardizing the hydrochloric acid solution, draw off exactly 25 cubic centimeters of the previously standardized $\frac{N}{2}$ HCl and place in a clean 200-cubic centimeter Erlenmyer flask, add approximately 50 cubic centimeters neutral distilled water, and 2 to 3 drops of alazarin red indicator. Fill the alkali burette with the solution to be standardized and titrate until the original yellow color of the solution has disappeared and has been replaced with a brown (neutral point) but has not turned red. When the brown point has been reached ½ drop in excess will cause a decided change of color. Take the reading at the neutral point. Repeat until the relation between the two solutions has been definitely determined. The readings in all cases should be less than 25. Readings of more than 25 indicate that the alkali solution is too weak and must be made stronger before going further.

Calculations.—Assume that 23.7 cubic centimeters of the hydroxide solution are required to neutralize exactly 25 cubic centimeters of the standardized $\frac{N}{2}$ HCl solution and that the original volume was 5,000 cubic centimeters and that 175 cubic centimeters have been withdrawn for testing purposes. Then the volume to which the hydroxide solution should be made in order to rende it $\frac{N}{2}$ may be calculated as follows:

$$25 : 23.7 :: x : 4{,}825$$
$$x = 5{,}089.$$

5,089 − 4,825 = 264 cubic centimeters of neutral water that must be added to make the solution exactly $\frac{N}{2}$ for the particular burette used. Titrate the corrected alkaline solution against the standardized acid. The readings must be exactly equal. This hydroxide solution and its corresponding burette may be used in the standardization of other acid solutions.

Oxidizing and Reducing Solutions

The term *oxidation*, in its narrowest sense, signifies the taking up of oxygen by an element or compound, if the oxygen is secured from another compound that compound is reduced. Advantage is taken of this fact in the estimation and determination of iron, calcium, manganese, etc. For instance, ferrous iron may be

determined by adding standard potassium permanganate to a solution of the salt in the presence of an excess of sulfuric acid. the reaction proceeding as follows:

$$2KMnO_4 + 10FeSO_4 + 8H_2SO_4 = 5Fe_2(SO_4)_3 + K_2SO_4 + 2MnSO_4 + 8H_2O.$$

As a potassium permanganate solution is practically the only one of this class required in soil determinations. the discussion will be limited to its preparation and standardization.

Preparation of a $\frac{N}{10}$ Potassium Permanganate Solution.—A $\frac{N}{10}$ potassium permanganate solution is one in which enough oxygen is contained to oxidize $\frac{1}{10}$ gram of hydrogen in 1,000 cubic centimeters of solution. As the potassium permanganate in acid solution is assumed to split into the oxides of the metals. with liberation of oxygen. according to the reaction.

$$2KMnO_4 = K_2O + 2MnO + 5O$$

it is seen that 2 molecules yield 5 atoms of oxygen which in turn is equivalent to 10 atoms of hydrogen. In other words. $\frac{1}{50}$ of a gram-molecule of potassium permanganate (3.161 grams) is required for 1,000 cubic centimeters of a $\frac{N}{10}$ solution. It is not advisable to use a solution prepared in this manner as the water used usually contains traces of organic matter oxidizable by the permanganate. Consequently 3.2 to 3.3 grams of the salt is diluted to 1,000 cubic centimeters. and allowed to stand 8 to 10 days. filtered through an ignited asbestos filter and then standardized.

Standardization of $\frac{N}{10}$ Potassium Permanganate

As sodium oxalate can be purchased in a very pure condition, a solution of this salt is used for standardizing the permanganate solution. Place approximately 10 grams of the salt in an oven and heat at 130°C. for 2 hours to drive off all moisture. and cool in a desiccator. Weigh out exactly 6.700 grams of the dried salt and dilute to exactly 1,000 cubic centimeters. This solution is $\frac{N}{10}$ sodium oxalate.

Now calibrate and standardize two 50-cubic centimeter burettes and fill one with the $\frac{N}{10}$ sodium oxalate solution and the

other with the permanganate solution. Run exactly 50 cubic centimeters of the oxalate solution into a 250-cubic centimeter Erlenmeyer flask, add about 10 cubic centimeters of double normal sulfuric acid solution, heat to appproximately 70°C. and titrate with the permanganate. No indicator is necessary as the permanganate acts as the indicator. The titration should proceed slowly, especially at the start, add a drop of the permanganate, agitate the flask and wait for the disappearance of color. After the reaction has started, the permanganate may be added rapidly. Titrate until the solution remains pink for ½ minute after a thorough shaking. From the results secured, calculate the strength of the permanganate, and the amount of dilution necessary to make exactly $\frac{N}{10}$ as directed under "Dilution of approximately $\frac{N}{2}$ HCl to exactly $\frac{N}{2}$," page 63.

The Preparation of Indicators.—The following indicators, in addition to those given under hydrogen-ion concentration, are needed in every fertility laboratory.

Alazarin Red

(pH 5.0–6.8)

Alkaline, red. Neutral, brown. Acid, yellow.

Dissolve 1 gram of the salt in 100 cubic centimeters of 60 per cent alcohol, or 1 gram in 100 cubic centimeters of neutral water.

Used in all determinations involving the titration of ammonia, except where aluminum is used, (the reduction method).

Cochineal

(pH 4.8–6.2)

Alkaline, violet. Acid, yellowish red.

Extract 3 grams of the macerated material with 50 cubic centimeters of alcohol, add 200 cubic centimeters of pure water and allow to stand for 1 week, with frequent shaking. Decant the supernatant liquid.

Used in total nitrogen determinations, titration of ammonia, where sulfides or sulfur obscures the end-point with other indicators.

Methyl Orange

(pH 3.1–4.4)

Alkaline, yellow. Acid, red.

Dissolve 0.2 gram of the acid salt, or 0.22 gram of the sodium salt in 100 cubic centimeters of pure water. If the sodium salt is used, add 0.67 cubic centimeter of $\frac{N}{1}$ hydrochloric acid, allow to settle and filter.

Used in testing phosphorus washings and in the titration of carbon-dioxide.

Methyl Red

(pH 4.2–6.3)

Alkaline, yellow. Netural, brown. Acid, red.

Dissolve 2 grams of the salt in 1,000 cubic centimeters of 95 per cent alcohol.

This indicator is used the same as alazarin red.

Phenolphthalein

(pH 8.3–10.0)

Alkaline, red. Acid, colorless.

Dissolve 10 grams of the salt in 1,000 cubic centimeters of 86 per cent alcohol.

Used in titrating weak alkali, carbon-dioxide determinations, phosphorus determinations, etc. Useless for the determination of ammonia. Carbon-dioxide acts as an acid toward this indicator.

Rosolic Acid

(pH 6.9–8.0)

Alkaline, rose red. Acid, yellow.

Dissolve 10 grams of the salt in 1,000 cubic centimeters of 60 per cent alcohol.

Used for titrating ammonia when aluminum has been used (the reduction method). Affected by large amounts of ammonium

salts, consequently $\frac{N}{X}$ ammonium hydroxide solution should not be used as the alkali in the titration.

Thymol Blue

(pH 8.0—9.6)

Alkaline, blue. Neutral, muddy green. Acid, lemon yellow.

Place 1 decigram of the salt in a 250-cubic centimeter Florence flask; add 4.3 cubic centimeters $\frac{N}{20}$ sodium hydroxide, and bring into solution by placing the flask into hot water and agitating until solution is complete. When solution is complete build up to 250 cubic centimeters with neutral water.

Used as a substitute for phenolphthalein in the titration of carbon-dioxide.

Brom Phenol Blue

(pH 2.8–4.6)

Alkaline blue. Neutral, muddy green. Acid, lemon yellow.

Place 1 decigram of the salt in a 250-cubic centimeter Florence flask, add 3.0 cubic centimeters $\frac{N}{20}$ sodium hydroxide and bring into solution by placing the flask in hot water. When solution is complete build up to 250 cubic centimeters with neutral water.

Used as a substitute for methyl orange in the titration of carbon-dioxide.

HYDROGEN-ION CONCENTRATION

Extensive studies have shown that the measurement of hydrogen-ion concentration of soils is necessary for explaining many phenomena associated with the various conditions found.

The Determination of Hydrogen-ion Concentration:

The determination of hydrogen-ion concentration is based on the measure of the intensity or strength of the acid ion (hydrogen) or basic ion (hydroxyl) in solution. The strength depends upon the degree of dissociation of the compound in solution. The solution containing the dissociated ion is placed on

the same basis as the normal solution, hence we have a normal hydrogen-ion solution.

A Normal Hydrogen-ion Solution is one that contains one gram of hydrogen, in the ionized state in 1,000 cubic centimeters of solution.

Expression of Hydrogen-ion Concentration.—Water dissociates slightly into H^+ and OH^- ions; $H_2O = H^+ + OH^-$. In pure distilled water at 22°C. the concentration (C) of H^+ and OH^+ ions are equal in number and consequently are constant. The value of this constant has been found to be 10^{-14}. In pure water the hydrogen-ion constant C_{H+}, and the hydroxyl-ion constant C_{OH-} each have the value of 10^{-7} for either kind of ion. In other words, the amount of ionized hydrogen in pure water at 22°C. has been found by electrical measurements to be $\frac{1}{10,000,000}$ gram per liter. The use of this unwieldy fraction may be avoided by writing the expression 10^{-7}. As H^+ ion concentration (P_H value) are always expressed by an exponent to the base 10, this base need not be indicated, and by common consent the minus sign of the exponent is also dropped; thus the H^+ ion concentration of pure water is expressed by the figure 7.0 which is known as its P_H value. The P_H is actually the logarithm of the reciprocal of the H^+ ion concentration. Thus in pure distilled water the concentration of H ion is $\frac{1}{10,000,000}$ gram per liter. The reciprocal of $\frac{1}{10,000,000}$ is 10,000,000, the common logarithm of which is 7. This is the P_H for distilled water.

Measurement of Hydrogen-ion Concentration. *Electrometric Method.*—The only accurate measurement of hydrogen-ion concentration is by the electrometric method. The method is based on a determination of the electromotive force of the gas chain of which the solution under test is one of the components. The manipulation requires the use of a special apparatus usually of a delicate nature. The determination, however, is very accurate and may be carried to a thousandth of a degree. As special instructions are furnished by the manufacturers with each set, no attempt will be made to describe the manipulations. The reader is referred to the standard works for particulars.

Colorimetric Determination of Hydrogen-ion Concentration Single Color Standards.—For routine work, the use of certain indicators, which give definite end points or color changes at different points of the scale of P_H values, will give the desired

and sufficiently accurate results. The indicators in general use are those of Clark and Lubs. The list of indicators with their color range follows:

TABLE 2.—CLARK AND LUB'S LIST OF INDICATORS

Chemical name	Common name	Color change	P_H range	Cubic centimeters $\frac{N}{20}$ NaOH per decigram
Thymol sulfon phthalein (acid range)	Thymol blue	Red to yellow	1.2–2.8	4.3
Tetra bromo phenol sulfon phthalein	Brom phenol blue	Yellow to blue	3.0–4.6	3.0
Ortho carboxy benzine azo di methyl aniline	Methyl red	Red to yellow	4.4–6.0	7.4
Di bromo ortho cresol sulfon phthalein	Brom cresol purple	Yellow to purple	5.2–6.8	3.7
Di bromo thymol sulfon phthalein	Brom thymol blue	Yellow to blue	6.0–7.6	3.2
Phenol sulfon phthalein..	Phenol red	Yellow to red	6.8–8.4	5.7
Ortho cresol sulfon phthalein	Cresol red	Yellow to red	7.2–8.8	5.3
Thymol sulfon phthalein (alkaline range)	Thymol blue	Yellow to blue	8.0–9.6	4.3
Ortho cresol phthalein....	Cresol phthalein	Colorless to red	8.2–9.8	0.0

Preparation of Indicators.—One decigram (0.1 gram) of the dry powder is ground in an agate mortar with the amount of $\frac{N}{20}$ NaOH indicated above. When solution is complete, dilute to

25 cubic centimeters. This gives a 0.4 per cent solution of the original dye. Ten cubic centimeters of this stock solution are further diluted to 100 cubic centimeters, thus giving a concentration of 0.04 per cent. Cresol phthalein and phenol phthalein and, in some cases, methyl red are used in a 0.04 per cent solution of 95 per cent alcohol. Five drops of indicators so prepared, will be found satisfactory for use in testing 10 cubic centimeters of soil solution, media, etc.

Preparation of Solutions of a Definite Hydrogen-ion Concentration.—The hydrogen-ion concentration of any unknown solution is determined colorimetrically by comparing the color produced in that solution by a certain indicator or series of indicators with the color produced by the same indicator or series of indicators in solutions, whose hydrogen-ion concentration is definitely known. The standard solutions are called buffer solutions. The buffer solutions recommended by Clark and Lubs may be accurately reproduced. The solutions are made by dilution from the following stock solutions using conductivity water in all cases:

$\frac{M}{5}$ potassium chloride + $\frac{M}{5}$ hydrochloric acid

$\frac{M}{5}$ acid potassium phthalate + $\frac{M}{5}$ hydrochloric acid

$\frac{M}{5}$ acid potassium phthalate + $\frac{M}{5}$ sodium hydroxide

$\frac{M}{5}$ acid potassium phosphate + $\frac{M}{5}$ sodium hydroxide.

$\frac{M}{5}$ boric acid; $\frac{M}{5}$ potassium chloride mixture + $\frac{M}{5}$ sodium hydroxide.

Preparation of Solutions.—These solutions must be prepared very carefully. All glassware must be free of any soluble material. The following directions are taken from Clark, "The Determination of Hydrogen Ions," pp. 100 to 107, 1923.

$\frac{M}{5}$ *Potassium Chloride Solution.*—Dilute 14.912 grams of pure dry KCl to 1,000 cubic centimeters. The salt should be recrystallized 3 or 4 times and dried at 120°C. for 2 days.

$\frac{M}{5}$ *Acid Potassium Phthalate Solution.*—Dissolve about 60 grams of potassium hydroxide in about 400 cubic centimeters of water, add 50 grams of commercial resublimed phthalic anhydrid,

if acid add more KOH. When roughly adjusted to a slight pink, add as much more phthalic anhydrid as the solution contains, and heat until all is dissolved. Filter while hot and allow the crystallization to take place slowly. The crystals should be drained with suction and recrystallized at least twice from distilled water. Do not allow crystallization to take place below 20°C. Dry the crystals at 110 to 115°C. to constant weight. Dilute 40.836 grams to 1,000 cubic centimeters for a $\frac{M}{5}$ solution.

$\frac{M}{5}$ *Acid Potassium Phosphate Solution.*—Dilute 27.232 grams of the pure salt, dried to constant weight at 110 to 115°C. to 1,000 cubic centimeters. The salt should be recrystallized, at least 3 times, from distilled water. The solution should be distinctly red with methyl red and distinctly blue with brom phenol blue.

$\frac{M}{5}$ *Boric Acid,* $\frac{M}{5}$ *Potassium Chloride.*—Dilute 12.4048 grams of boric acid and 14.912 grams of potassium chloride to 1,000 cubic centimeters. The boric acid should be recrystallized 2 to 3 times from distilled water. It should be air-dried in thin layers between filter paper, then to a constant weight in thin layers in a desiccator over $CaCl_2$.

$\frac{M}{5}$ *Sodium Hydroxide Solution.*—Dissolve 100 grams of NaOH in 100 cubic centimeters distilled water in a Pyrex flask. Cover the mouth of the flask with tinfoil and let stand overnight to allow the carbonates to settle. If the solution is made several months in advance, the carbonates will settle out leaving a clear concentrated solution. In this case, the clear solution is carefully pipetted off. Usually it is necessary to free the solution of the suspended carbonates. The following procedure is recommended: Fit a hardened filter paper to a Buchner funnel. Remove the paper from the funnel and treat it with a warm, 1 : 1 sodium hydroxide solution. Allow to stand a few minutes, then decant the solution and wash the paper, first with absolute alcohol, then with dilute alcohol, and then with large quantities of distilled water. Replace the wet paper in the funnel and gently apply suction until the greater part of the water has evaporated. Do not allow the paper to become so dry that it curls. Pour the concentrated alkali upon the middle of the paper, spread it

with a glass rod, making sure that the paper, under gentle suction, adheres well to the funnel, and allow the suction to draw the solution through. Dilute the filtrate quickly with conductivity water to a solution somewhat more concentrated than $\frac{N}{1}$. Determine the strength of this solution by titration with an acid of known strength, and calculate the dilution necessary to make the final solution $\frac{M}{5}$. Make all dilutions with the least possible exposure and keep the final dilution in a bottle that has been thoroughly paraffined. A calibrated 50-cubic centimeter burette should be attached to this bottle, and should be protected from contamination by suitable soda-lime guards. The solution should be carefully standardized with weighed quantities of acid potassium phthalate or with the $\frac{M}{5}$ solution. Weigh out carefully about 1.6 grams of the purified salt, dissolve in about 20 cubic centimeters of water, and add 4 drops of phenol phthalein. Pass a stream of CO_2 free air through the solution and titrate with the alkali until a faint but distinct and permanent pink is developed. Do not attempt to adjust the solution accurately, as a factor is to be preferred to the possible contamination with CO_2.

$\frac{M}{5}$ *Hydrochloric Acid Solution.*—Dilute a high-grade hydrochloric acid to about 20 per cent and distill. Dilute to approximately $\frac{M}{5}$ and standardize with the sodium hydroxide solution. The solution may be further standardized with silver chloride and thus serve as a check on the standard alkali.

TABLE 3.—COMPOSITION OF MIXTURES GIVING P_H VALUES AT INTERVALS OF 0.2 WHEN AT 20°C.

KCl—HCl Mixtures

(Given by Clark and Lubs as preliminary measurements)

P_H values	Cubic centimeters $\frac{M}{5}$ KCl	Cubic centimeters $\frac{M}{5}$ HCl	Diluted to cubic centimeters
1.2	50	64.5	200
1.4	50	41.5	200
1.6	50	26.3	200
1.8	50	16.6	200
2.0	50	10.6	200
2.2	50	6.7	200

Phthalate—HCl Mixtures

P_H values	Cubic centimeters $\frac{M}{5}$ potassium acid phthalate	Cubic centimeters $\frac{M}{5}$ hydrochloric acid	Diluted to cubic centimeters
2.2	50	46.70	200
2.4	50	39.60	200
2.6	50	32.95	200
2.8	50	26.42	200
3.0	50	20.32	200
3.2	50	14.70	200
3.4	50	9.90	200
3.6	50	5.97	200
3.8	50	2.63	200

Phthalate—Sodium Hydroxide Mixtures

P_H values	Cubic centimeters $\frac{M}{5}$ potassium acid phthalate	Cubic centimeters $\frac{M}{5}$ sodium hydroxide	Diluted to cubic centimeters
4.0	50	0.40	200
4.2	50	3.70	200
4.4	50	7.50	200
4.6	50	12.15	200
4.8	50	17.70	200
5.0	50	23.85	200
5.2	50	29.95	200
5.4	50	35.45	200
5.6	50	39.85	200
5.8	50	43.00	200
6.0	50	45.45	200
6.2	50	47.00	200

Acid Potassium Phosphate—Sodium Hydroxide Mixtures

P_H values	Cubic centimeters $\frac{M}{5}$ KH_2PO_4	Cubic centimeters $\frac{M}{5}$ NaOH	Diluted to cubic centimeters
5.8	50	3.72	200
6.0	50	5.70	200
6.2	50	8.60	200
6.4	50	12.60	200
6.6	50	17.80	200
6.8	50	23.65	200
7.0	50	29.63	200
7.2	50	35.00	200
7.4	50	39.50	200
7.6	50	42.80	200
7.8	50	45.20	200
8.0	50	46.80	200

Boric Acid and Potassium Chloride—Sodium Hydroxide Mixtures

P_H values	Cubic centimeters $\frac{M}{5}$ H_3BO_3 $\frac{M}{5}$ KCl mixture	Cubic centimeters $\frac{M}{5}$ NaOH	Diluted to cubic centimeters
7.8	50	2.61	200
8.0	50	3.97	200
8.2	50	5.90	200
8.4	50	8.50	200
8.6	50	12.00	200
8.8	50	16.30	200
9.0	50	21.30	200
9.2	50	26.70	200
9.4	50	32.00	200
9.6	50	36.85	200
9.8	50	40.80	200
10.0	50	43.90	200

The consistency of the 5.8 and the 6.2 phthalate should be compared with the same P_H concentration of the phosphate using bromocresol purple, also the 7.8 and 8.0 phosphate should be compared with the same P_H of the borates using cresol red.

Comparison of Colors.—The determination is made by placing 10 cubic centimeters of the solution to be examined in a test tube and adding 5 drops of the proper indicator. Several trials may be necessary to determine the proper indicator to be used. The color produced is then compared with that of standards of a known P_H value. It is important that the same size tubes are used throughout all the work. This method will allow the worker to approximate a P_H value within 0.1 degree which is sufficiently accurate for all ordinary work.

Color Comparator.—In case there is difficulty in determining the exact depth of color or if there is a slight cloudiness in the solution, the comparisons may be made in a color comparator. This is an ordinary block of wood with six deep holes slightly larger than the test tubes. The holes are bored in pairs and are parallel to each other. The pairs of holes are placed as close to each other as possible, without breaking the separating walls.

Smaller holes are bored through the sides of the block 1 to 1½ inches from the top so that they will pass through each pair of larger holes, in order that the test tubes may be viewed through the sides. The center pair of test tubes hold, first, the solution to be tested with the indicator in it; and second, a water blank or the solution to be tested without any indicator. The standards are placed on either side of the center and are backed with blanks of the solution to be tested. The whole block, and especially the holes, should be painted or dyed a non-reflecting black before use.

Permanent color standards may be prepared from the standard solutions in the ordinary way. Mold growth is prevented by a drop of toluol. The tubes should be stoppered with corks protected with tinfoil and sealed with paraffine. If they have been made for some time these permanent color standards should always be used with caution as they may change slightly. Methyl red especially is subject to change.

Double Color Standards.—It is frequently advantageous to use the double color standards for the determination of P_H values, especially in cases where there is any question regarding the amount of light passing through, as in turbid solutions,[23] soil extracts,[15] etc. This is also the case where a few determinations are to be made, and one does not have the time to make the carefully prepared solutions that have been described.

Preparation of Buffer Solutions:[18]

1. Normal acetic acid 57.7 cubic centimeters of glacial acetic acid diluted to 1,000 cubic centimeters. Used for the acid colors of bromophenol blue and methyl red.

2. Dilute 7.0 grams of potassium dihydrogen phosphate (KH_2PO_4) to 1,000 cubic centimeters with neutral water. Used for the acid colors of bromocresol purple, bromothymol blue, phenol red, cresol red, and thymol blue (alkaline range).

3. Dilute 18.0 grams of disodium phosphate dodecahydrate ($Na_2HPO_4.2H_2O$) to 1,000 cubic centimeters with neutral water. Used for the alkaline colors of bromophenol blue and methyl red and also for bromocresol purple, and bromothymol blue, if desired.

4. Dilute 1.0 gram of anhydrous sodium carbonate to 1,000 cubic centimeters with neutral water. Used for the alkaline

colors of bromocresol purple, bromothymol blue, phenol red, cresol red, and thymol blue (alkaline range).

Indicators.—The Clark and Lubs series of indicators are used throughout the determination. They may be prepared according to the directions given above using 4 drops to produce the color, or better still, they may be prepared according to the table given below, and diluted to the required concentration (250 cubic centimeters). The indicators, both the stock and diluted solutions, should be kept in dark-colored bottles. The bottles containing the diluted solutions should be stoppered with a stopper fitted with a 1-cubic centimeter pipette which is always used in connection with that particular indicator. In the case of methyl red, the indicator is dissolved in 150 cubic centimeters of alcohol and diluted to 250 cubic centimeters.

Preparation of Color Standards:

1. Place 11 pairs of test tubes of uniform diameter in a rack or in a block fitted with holes to receive them.
2. Add the correct number of drops of indicator to each tube.
3. Add the correct number of drops of buffer solution to total 10 drops to each tube, making exceptions as noted below.
4. Add the proper amount of buffer to each tube from a burette.
5. Preserve the solutions by adding 2 or 3 drops of toluene to each tube.
6. Stopper the tubes with paraffine corks. As methyl red is decolorized by toluene, stopper loosely for several days in order that the excess toluene may evaporate.

The amounts of indicator, buffer, and the P_H value of each pair of tubes are given in Table 4 on following page.

TABLE 4

Drops of indicator in		Drops of buffer to equalize level in tubes		P_H values of each pair of tubes						
Alkali tube	Acid tube	Alkali tube	Acid tube	Bromophenol blue	Methyl red	Bromocresol purple	Bromothymol blue	Phenol red	Cresol red	Thymol blue
1	9	9	1	3.1	4.05	5.3	6.15	6.75	7.15	7.85
3[1]	17[1]	17[1]	3[1]	3.3	4.25	5.5	6.35	6.95	7.35	8.05
2	8	8	2	3.5	4.4	5.7	6.5	7.1	7.5	8.2
3	7	7	3	3.7	4.6	5.9	6.7	7.3	7.7	8.4
4	6	6	4	3.9	4.8	6.1	6.9	7.5	7.9	8.6
5	5	5	5	4.1	5.0	6.3	7.1	7.7	8.1	8.8
6	4	4	6	4.3	5.2	6.5	7.3	7.9	8.3	9.0
7	3	3	7	4.5	5.4	6.7	7.5	8.1	8.5	9.2
8	2	2	8	4.7	5.6	6.9	7.7	8.3	8.7	9.4
17[1]	3[1]	3[1]	17[1]	4.8	5.75	7.0	7.85	8.45	8.85	9.55
9	1	1	9	5.0	5.95	7.2	8.05	8.65	9.05	9.75
Note 1. Adjust the indicator solution to a percentage of..				0.008	0.008	0.012	0.008	0.004	0.008	0.008
Note 2. Cubic centimeters of $\frac{N}{10}$ NaOH per 0.1 gram of indicator for stock solution.				1.64	Alcoholic solution	2.78	1.77	3.10	2.88	2.38
Note 3. Produce acid color with 5 cubic centimeters of buffer number.....				1	1	2	2	2	2	2
Note 4. Produce alkaline color with 5 cubic centimeters of buffer number....				3	3	4	4	4	4	4

[1] Use 10 cubic centimeters of buffer instead of 5 to each tube.

Preparation of Unknown.—To 5 cubic centimeters of the unknown add 10 drops of indicator and compare with the standards in the color comparator compensating for color and turbidity.

The Color Comparator.—A block similar to that described above is used, the only difference being that the block is larger and contains nine holes arranged in sets of three, with the smaller side holes passing through each set.

The unknown is placed in the first hole of the center set and the other two holes are fitted with tubes containing distilled water. The pairs of standards are then placed, on each side of the unknown. A tube containing the unknown, without an indicator, is placed in each of the vacant holes, and the colors compared.

THE SOIL SOLUTION

Plants are dependent upon the compounds that are soluble in the water of the soil for their supply of mineral constituents. As an unbalanced supply of the mineral constituents or the presence of some interfering or toxic compound in solution may markedly affect plant growth, a study of the soil solution is frequently necessary in order to partially explain some of the phenomena of soil fertility and its related subjects. As it is practically impossible to separate the film surrounding the soil particle from the particle itself, a number of methods for obtaining the soil solution have been suggested.[27]

Methods for Obtaining the Soil Solution.—The methods commonly used for obtaining the soil solution are:

1. Extraction of the soil with an excess of water.
2. Displacement of the soil solution.

The solutions obtained by either of these processes lend themselves readily to the determination of nitrates and total salts.

Extraction.—Shake 100 grams of air-dry soil, or its equivalent of moist soil for 4 hours, with 500 cubic centimeters of distilled water, or allow to stand for 2 days with occasional shaking. Filter by means of a Buchner funnel, using suction, or by the use of a Pasteur-Chamberlain filter that has been washed free of all soluble material. Obtain a clear filtrate.

Displacement.—Place a known amount of soil in a tube as directed in the determination of capillary moisture, (Sec. 2, page 28). The tube should have a layer of sand in the bottom before the soil is placed in position. Place the tube upright in a ring stand and maintain a 2- or 3-inch layer of 95 per cent ethyl alcohol over the soil. The alcohol will flow down through the soil displacing and concentrating the soil solution which will drip from the bottom. Allow to drip until the odor of alcohol is noticed. No filtration of the solution is necessary. Thirty-five per cent or more of the soil solution may be extracted by this method, the amount depending upon the amount of moisture present.

Decolorizing the Soil Solution.—A colorless solution is not always obtained from the soil. As this color will interfere in calorimetric or turbidity determinations, it must be removed. The removal may prove a difficult process, due to the fact that the material capable of removing the color will absorb the elements to be determined. Usually the color will be removed by

the addition of 2 to 3 grams of carbon black, especially the brand "G Elf" (*Jour. Amer. Chem. Soc.*, 26, 811, 1904) or by the addition of 2 to 10 cubic centimeters of aluminum cream.

Aluminum Cream.—The aluminum cream is prepared according to the Standard Methods of Water Analysis[1] as follows: Dissolve 125 grams of sodium or potassium alum in 1,000 cubic centimeters of water, add ammonium hydroxide until all the aluminum hydroxide is precipitated, wash by decantation until free of chlorine. Usually all ammonia and nitrates will have disappeared before this time. The process will take a week to 10 days.

As 3 to 4 weeks are required to wash the aluminum hydroxide free of sulfates, it is advisable to precipitate the hydroxide from aluminum chloride, instead of alum, if the cream is to be used for decolorizing solutions in which sulfates are to be determined by the turbidity method.

If a cream-separator is available, the precipitate may be quickly washed by passing through the apparatus several times. The gelatinous mass adhering to the parts is washed off in distilled water and again passed through. The final washing is by decantation.

Analysis of the Soil Solution.—The analysis of the soil solution usually consists of a determination of the total salts, (see below). If the various elements, N.P.K.S. etc., are to be determined, the investigator is referred to the method given for each element. In case the various elements are to be determined it is usually advisable to increase the quantity of soil and water.

Determination of Total Salts:

Evaporate an aliquot of the soil solution to dryness in a tared crucible or beaker and heat to constant weight at a temperature of 110 to 120°C. Calculate in parts per million.

Estimation of the Concentration of the Soil Solution

The concentration of soluble salts in the soil solution may be estimated by the freezing point method of Bouyoucas.[4, 5] The method is based on the assumption that, as the soil solution contains soluble material its freezing point will be lowered according to its concentration. The method requires the use of a specially constructed piece of apparatus.

The Apparatus.—Place a 2-gallon, glazed earthenware jar inside a 4-gallon jar and pack the space between the two with

asbestos. Waterproof the upper part of the asbestos with paraffine. Arrange a cover for the inside jar with a $1\frac{1}{2}$-inch hole in the center and two $\frac{1}{4}$-inch holes, one on each side of the center hole. The large hole is fitted with a glass tube $1\frac{1}{2}$ inches in diameter and 6 inches long, whose top is flush with the top of the cover. This tube acts as an air-jacket and is fitted with a small tube, 1 inch in diameter and 9 inches long, extending almost to the bottom of the larger tube and held in place with a rubber stopper. The smaller tube is to contain the soil or the soil solution, hence is fitted with a Beckmann thermometer and is also secured with a stopper. The thermometer should cover a range of 5 or 6°C. and be graduated in $\frac{1}{100}$°C. so that by the aid of a lens it can be read to $\frac{1}{1,000}$ of a degree.

Determinations of the Freezing of Soils:

Use approximately 10 grams of peat, 20 grams of loams, silts, and clays, and 30 grams of sand, and thoroughly moisten with distilled water. Place enough of the moist sample in the 1-inch tube to make a column of soil about 1 inch. Fill the bath (inside jar) with crushed ice and add enough salt to produce a temperature of -3 to -4.5°C.

Set the thermometer so that the mercury thread will come to rest toward the upper part of the scale when the bulb is held in a mixture of pure ice and distilled water. Determine the freezing point of distilled water by placing a small amount of water in a tube, inserting the thermometer, and placing in the brine. Agitate the water by means of the thermometer until the freezing point is reached. Use this point as the standard for ascertaining the freezing point of the soil.

Insert the thermometer into the column of soil until the bulb is covered and place directly in the brine, and supercool to -1°C. Start solidification by holding the tube with one hand and gently moving the thermometer with the other. Immediately upon the commencement of solidification, as indicated by the rise in temperature, the tube is taken out of the cooling mixture and placed in the air-jacket. The temperature is allowed to rise until it comes to rest at a certain point on the scale and remains at that point for some time. The maximum temperature obtained is considered as the freezing point of the soil sample and is so recorded.

Estimation of Soil Colloids.—The amount of colloidal material in the soil may be estimated, if not determined, by the method (unpublished) developed by D. V. Moses of the Chemical Engineering Department, Iowa State College. The method is based upon the fact that the free water in the soil will freeze and expand, at a temperature not lower than −4°C., while the unfree water will not. As the colloidal material renders the soil water unfree, a determination of the amount of unfree water may be a determination of the colloidal matter.

The Apparatus.—(1) The apparatus consists of a 75- to 100-cubic centimeter Erlenmyer flask equipped with a two-holed rubber stopper. In one of the holes insert a thermometer, in the other insert a 2-cubic centimeter Mohr pipette, graduated in $\frac{1}{10}$ cubic centimeter. The tip of the pipette should be flush with the bottom of the stopper. The thermometer should be of such a length that the bulb will extend to approximately $\frac{3}{4}$ the distance to the bottom of the flask, yet will allow a reading of −7 to −10°C. above the top of the stopper.

2. Freezing bath as used in the freezing point determinations.

Analytical Process.—Weigh out two 100-gram portions of moist soil. Place one portion in the drying oven and dry overnight at a temperature of 110°C. Determine the total amount of water in the sample. If the sample is air dry, add 20 cubic centimeters distilled water, stopper and allow sufficient time for the moisture to become equally distributed through the mass. The process may be hurried by placing the stoppered flask in boiling water for a few minutes, then cooling.

Fill the flask approximately three-fourth full with gasoline. Place in a desiccator equipped with a cock for releasing the vacuum. Connect with a suction pump and apply suction until bubbling ceases. Fill the flask to such a height that when the stopper, containing the pipette and thermometer, is inserted tightly, the liquid will rise slightly in the pipette.

Place the flask in the cooling bath and (without agitation) cool slowly to −2 or −4°C. When 0°C. is reached, the height of the column must be closely watched as some soils will freeze at −2 or −3°C. Note the height of the column in the pipette at the lowest point after 0°C. is reached. If −4°C. is reached, without any change in the column, tap the flask to start freezing and adjust the temperature to the same point at which minimum reading was taken. Again read the pipette and note the height

of the column. The difference between the minimum and the maximum reading gives the volume expansion of the water in the soil.

Calculations.—Calculate the amount of unfree water by the use of the following formula:

$$M \times 1/10 - Y = X$$

M = total grams of water in the soil.
Y = volume expansion in cubic centimeters.
X = unfree water.

This formula assumes that 1 gram of distilled water, when frozen, expands $1/10$ cubic centimeter. Moses finds that 1 gram of collodial material will render 0.61 cubic centimeter of water unfree. The amount of unfree water divided by the factor 0.61 gives the amount of collodial material in the sample.

Plot on chart, using the cubic centimeter of unfree water as the ordinate, and the per cent of colloids (Bureau of Soils Method, Sec. 1, page 18) as the abscissa.

SOIL ACIDITY

The measurement of the acid condition of the soil can only be determined in one way, namely, by a determination of the hydrogen-ion concentration. There are, however, a great many methods that are in use for the practical measurement of acidity, (Lime Requirement Methods), a few of which are given below.

Lime Requirement Methods.—Lime requirement methods make use of various soil phenomena as follows:

Veitch Method.—The lime requirement measured by the addition of varying amounts of dilute lime water and determining the amount that just neutralizes the soil.

Hopkins Method.—The lime requirement measured by the action of soil acids on potassium nitrate.

Jones Method.—The lime requirement measured by the action of soil acids on calcium acetate.

Truog Method.—The lime requirement measured by the action of soil acids on zinc sulfide.

Tacke Method.—The lime requirement measured by the action of soil acids on calcium carbonate.

Potassium Thiocyanate Methods.—The lime requirement measured by the action of soil acids on the iron content of the soil.

Veitch Lime-requirement Method[38,39]

This method is based upon the assumption that the soil acids will exactly neutralize standard lime water.

Reagents:

$\frac{N}{28}$ CaO free of CO_2 contamination.

Determination.—To 10-gram portions of air-dry soil placed in 250-cubic centimeter Erlenmyer flasks, add 50 to 60 cubic centimeters CO_2 free distilled water and different amounts of $\frac{N}{28}$ CaCO solution, 2-5-10-20-30 cubic centimeters, etc. Dry down at once on the steam bath. Do not hurry the drying process. Allow to dry overnight if necessary. To the dry soil, add 100 cubic centimeters CO_2 free water, stopper, shake thoroughly, and place in an inclined position overnight. Draw off 50 cubic centimeters of the clear solution, place in a beaker, add a few drops of phenolphthalein and boil until the appearance of a pink color develops, or to about 5 cubic centimeters if no color develops.

Repeat, using smaller differences, ½ to 1 cubic centimeter, the smallest amount of lime water, which gives the characteristic pink, is taken as the acid equivalent. Calculate the amount of lime required.

The procedure, temperature, time of drying, etc., must be exactly the same in all cases.

Calculations.—One cubic centimeter of $\frac{N}{28}$ CaO contains 0.002 gram CaO.

One acre of air-dry soil to a depth of 6⅔ inches weighs approximately 2,000,000 pounds, or 907,184,800 grams. If 1 cubic centimeter of $\frac{N}{28}$ CaO is required to neutralize 10 grams of soil, then $\frac{907,184,800}{10} \times 0.002 = 181,436.8$ grams that would be required to neutralize 1 acre. Converting to pounds, we find that $\frac{181,436.8}{453.6} = 400$ pounds CaO. Hence each cubic centimeter $\frac{N}{28}$ CaO used is equivalent to a lime requirement of 400 pounds calcium oxide.

Hopkins Lime-requirement Method[20]

This method is based upon the assumption that soil acids will replace and liberate the acid ion in a neutral salt.

Reagents:

$\frac{N}{1}$ potassium nitrate.

Determination.—Place 100 grams of soil in a 500-cubic centimeter shaker bottle and add 250 cubic centimeters of $\frac{N}{1}$ potassium nitrate and shake for 3 hours. Let stand overnight, draw off 125 cubic centimeters, boil 10 minutes to expel the carbon-dioxide and titrate the acid liberated with $\frac{N}{25}$ sodium hydroxide, using phenolphthalein as indicator. Repeat the treatment on the same sample until no more acid is produced in the last 125 cubic centimeters drawn off. The sum of all titrations represents the total acidity of 100 grams of soil.

Calculations.—As each cubic centimeter of the $\frac{N}{25}$ NaOH solution used contains the same amount of alkalinity as each cubic centimeter of a $\frac{N}{25}$ CaO solution, then each cubic centimeter of the alkali is equivalent to 0.00024 gram of CaO.

Calculate in pounds per acre as recommended in the Veitch determination.

Jones Lime-requirement Method[21]

This method is based on the same assumption as the Hopkins method.

Determination.—To 5.6 grams of soil add 0.5 gram of calcium acetate, mix well in a 3-inch mortar with pestle, add enough water to make a stiff paste. Pestle 30 seconds, add 30 cubic centimeters of water and mix again 30 seconds. Wash into a 200-cubic centimeter flask, keeping the bulk down to approximately 160 cubic centimeters. Let stand several hours, shaking every 15 minutes. Make volume to 200 cubic centimeters, shake, filter through a dry filter, discarding the first 10 to 15 cubic centimeters which may be cloudy. Titrate 100 cubic centimeters of clear filtrate, using $\frac{N}{10}$ NaOH with phenolphthalein. Two times the reading gives cubic centimeters of alkali required to neutralize the entire amount of solution. This result, times the factor 1.8 times 1,000 equals pounds of lime CaO required per 2,000,000 pounds of soil. The factor is tentative.

Truog Lime-requirement Method[36]

Based upon the fact that the soil acids will react with zinc sulfide, liberating hydrogen sulfide as a gas in a boiling solution. The escaping hydrogen sulfide is brought in contact with a paper containing lead acetate, the resulting formation of lead sulfide (black) causes a discoloration of the paper.

$$ZnS + H \text{ acid (soil acid)} = H_2S + Zn \text{ acid}$$
$$H_2S + Pb(C_2H_3O_2)_2 = PbS + 2CH_3COOH$$

Reagents:

Commercial Salt.—The most satisfactory results may be obtained by the use of the commercial salt (Truog reagent) sold for this purpose and which may be obtained from any chemical supply house.

Laboratory Preparation.—Prepare a suspension of 5 grams of zinc sulfide* [illegible] grams of Ca $Cl_2 2H_2O$ or 75 grams $CaCl_2 6H_2O$, in 250 cubic centimeters pure water. The solution must be neutral. Barium chloride may be used in place of calcium chloride. The solution must be thoroughly shaken and all zinc brought in suspension before use.

Determination.—Weigh out air-dry samples as follows, or use an equivalent amount of moist sample: Sand or sandy loam 12 grams; clay loam 10 grams; peat 2½ grams. Place in 250-cubic centimeter Erlenmyer flask, add 1 gram of the prepared commercial salt or 5 cubic centimeters of the above solution, and 100 cubic centimeters neutral distilled water. Shake thoroughly, place over a low carbon flame, and bring to boil. The flame should be so adjusted that the solution will take 4 to 5 minutes heating before boiling. Boil the solution 1 minute, then place a strip of filter paper moistened with 10 per cent lead acetate over the mouth of the flask and continue the boiling for exactly 2 minutes. The prepared filter paper is sold with the commercial salt, and may be used in the dry state. The color developed on the paper by the formation of PbS is indicative of the degree of acidity. (See color chart, plate I.)

*Neutral zinc sulphide prepared by precipitating ZnS from an aqueous solution (50 grams zinc acetate and 5 grams ammonium sulphate dissolved in 500 cubic centimeters pure water) by passing H_2S through. The precipitation throughout is made at a boiling temperature, the first few grams containing Fe., Pb. or Cd., being filtered and discarded. Wash on a Buchner funnel with hot water, dry and grind to a fine powder.

Tacke Lime-requirement Method[34]

The Tacke method is based upon the fact that the soil acids will combine with calcium carbonate with the evolution of carbon-dioxide. While this method is slow, it overcomes the objectionable feature of boiling that is necessary in the previous methods.

Reagents:

1. Approximately $\frac{N}{5}$ NaOH solution.

2. Pure calcium carbonate ground to a fine powder.

Determination.—Place 20 grams of air-dry soil, or its equivalent, in moist soil, in 300-cubic centimeter Kjeldahl flasks, add 2 or more grams of calcium carbonate and 75 to 100 cubic centimeters, CO_2 free distilled water. Connect as a member of the aeration unit and aerate slowly, absorbing the CO_2 evolved in 50 cubic centimeters of approximately $\frac{N}{5}$ NaOH diluted to 100 cubic centimeters. The alkali should be placed in a deep cylinder in order that the bubble will be exposed as long as possible to the absorbent. The flasks should be shaken throughout the determination. Aerate for 5 hours.

At the end of the aeration period determine the amount of CO_2 absorbed by titration. (See Titration of Carbon Dioxide, page 119.)

Calculate the lime requirement of the soil from the amount of dilute acid used in the second part of the titration, using the same method described in the Veitch determination.

Potassium Thiocyanate Lime-requirement Method

The use of 4 per cent potassium thiocyanate in alcohol as a test for soil acidity was recently suggested by Comber.[9] The test is based upon the assumption that a portion of the iron in an acid soil is in the *ic* state. The amount of ferric compounds is apparently dependent upon the degree of acidity. As potassium thiocyanate gives no reaction with the ferrous salts of the soil unless they are present in large amounts but with the ferric compounds produces the blood-red ferric thiocyanate,

$$FeCl_3 3KCNS = Fe(CNS)_3 3KCl,$$

the depth of color is taken as an indication of the degree of lime requirement. In case large amounts of ferrous salts are present

the color will be green. The Comber test can only be used in air-dry soils.

The Emerson Method

Emerson[13] has devised a method that is an improvement of the thiocyanate lime-requirement tests. It is adapted for moist soils, gives a more pronounced color, clarifies more rapidly, and is adapted for use under field conditions. The degree of color may be accurately determined by comparison with color standards or with color charts.

Reagents:

Solution No. 1.—A volume of ether saturated with neutral ammonium molybdate. If no application of phosphatic fertilizers has been made to the soils to be tested the neutral ammonium molybdate may be omitted.

Solution No. 2.—Dissolve 10 grams of potassium thiocyanate in a mixture of 900 cubic centimeters acetone and 100 cubic centimeters of ether. The solution should be corked tightly and kept in a dark place.

Determination.—Fill a hard glass, test tube (15 millimeters × 12.5 centimeters) approximately one-fourth full with the soil to be tested. Add 10 to 20 drops of solution No. 1 to remove any interfering phosphates. The amount to be added will vary according to the degree of moisture in the soil. Add just enough to coat the soil particles with a film of the solution. Do not saturate the soil with the solution. Now fill the tube approximately one-half full, with solution No. 2, cork tightly and shake vigorously in a horizontal position for 30 seconds. The soil will settle in 30 to 45 seconds. Determine the degree of acidity by comparison with the color standards below or with the chart, plate I facing page 88. If the soil is dry, the use of solution No. 1 may be omitted.

Preparation of Color Standards.—When the test is made in the field, it is desirable to carry the least possible amount of equipment. For this reason a color chart is used for purposes of comparison. This may be prepared on heavy paper, using water colors that are of the same shades as those of the standards below. Under laboratory conditions, a greater degree of accuracy is desirable, and solutions of varying degrees of density corresponding to the average color produced in the actual test may be pre-

pared. Such solutions may easily be made by using the common bacteriological stain "Safranin." This stain, however, produces a color that is too red and hence must be toned down to the desired brown tones by adding a caramel solution. The following method of making standard color solutions has been found to be very satisfactory:

Solution No. 1.—One gram of safranin is triturated in a mortar in a measuring flask with small amounts of pure water, until no more color is washed out and the whole made up to 250 cubic centimeters.

Solution No. 2.—One gram of caramel is triturated as in solution No. 1 and built up to 100 cubic centimeters.

Using the proportions indicated in the following table 500-cubic centimeter lots of varying standard color solutions are made up. The safranin solution is first measured into the flask and diluted with approximately three-fourth the required amount of water, next the caramel solution is added, then the alcohol and then water up to the mark. Whenever possible the use of conductivity water is recommended for the preparation of the standards. The alcohol is added merely as a preservative.

FORMULA FOR COLOR STANDARDS

Cubic centimeters Solution No. 1	Cubic centimeters water	Cubic centimeters 85 per cent alcohol	Cubic centimeters Solution No. 2	Approximate amount of lime to neutralize, tons per acre
125.0	295.0	50	30	5
62.5	362.5	50	25	4
31.2	398.8	50	20	3½
15.8	414.2	50	20	3
7.8	427.2	50	15	2½
3.9	431.1	50	15	2
1.95	438.1	50	10	1½
1.0	439.0	50	10	1
0.5	444.5	50	5	½

PROCEDURE FOR STUDENT DETERMINATION OF LIME REQUIREMENT

1. Determine the approximate acidity of the soil by either of the above methods excepting the Veitch, and calculate the

amount of $\frac{N}{28}$ CaO solution that will be required to neutralize 10 grams of the soil. For calculation see Veitch test.

2. Weigh out five 10-gram portions of this soil and place in 50-cubic centimeter evaporating dishes.

3. Letter the dishes and make additions of $\frac{N}{28}$ CaO as follows:

Dish Letters	Add Lime Water
a	5 cubic centimeters less than the calculated amount.
b	2.5 cubic centimeters less than the calculated amount.
c	exactly the calculated amount.
d	2.5 cubic centimeters more than the calculated amount.
e	5 cubic centimeters more than the calculated amount.

4. If the amounts added are not sufficient to moisten the soil thoroughly, add enough distilled water to make it moist.

5. Immediately place on steam bath and evaporate to dryness.

6. When dry transfer the soil to a 250-cubic centimeter Erlenmyer flask, add 100 cubic centimeters distilled, carbon-dioxide-free water, stopper, and shake every 5 minutes for 1 hour. Place in an inclined position and allow to stand overnight or until the next period.

7. Draw off 25 to 50 cubic centimeters of the clear solution, place in a 150-cubic centimeter beaker, add 5 drops phenolphthalein indicator and boil down to approximately one-third the original volume. The smallest amount of lime water giving the characteristic pink of the indicator is taken as the acid equivalent of that soil, and the amount of lime required may be calculated in the same manner as recommended for the Veitch lime-requirement method.

8. Repeat the process substituting some other indicator for phenolphthalein.

SOIL ALKALI

Soil alkalis are of three kinds: (1) white (sulfates and chlorides of sodium, magnesium, potassium, calcium, and bicarbonates of calcium); (2) black (carbonates and bicarbonates of sodium); (3) brown (nitrates of various bases). The acid ion is usually determined and calculated in terms of the most common salt. When necessary, the basic ion is determined in the usual manner.[32]

Determination of Carbonates:

Preparation of Solution.—Place 50 grams of the sample in shaker bottles, add 1,000 cubic centimeters of distilled water and shake 3 hours, allow to stand overnight, decant and filter.

Reagents:

Standard Potassium Hydrogen Sulfate Solution.—Dissolve 0.5580 gram of pure $KHSO_4$ in water and dilute to 1,000 cubic centimeters.

Analytical Process.—Place 100 cubic centimeters of the clear extract in a 300-cubic centimeter Erlenmyer flask, add a few drops of phenolphthalein and titrate to an exact disappearance of color.

$$Na_2CO_3 + KHSO_4 = KNaSO_4 + NaHCO_3.$$

Each cubic centimeter of the $KHSO_4$ solution is equivalent to 0.246 milligram of CO_3 or 0.1804 milligram of CO_2.

Determination of Bicarbonates:

Analytical Process.—After determining carbonates, add 2 to 3 drops of methyl orange indicator to the same solution, take the reading of the burette and continue the titration until the first reddish tinge appears.

$$NaHCO_3 + KHSO_4 = NaKSO_4 + H_2CO_3.$$

Each cubic centimeter of the $KHSO_4$ solution is equivalent to 0.25 milligram of HCO_3.

Determination of Chlorides:

Reagents:

1. *Standard Silver Nitrate Solution.*—Dissolve 2.3974 grams of pure silver nitrate in water and make up to 1,000 cubic centimeters.

2. *Potassium Chromate Solution.*—Make a saturated solution of pure potassium chromate for use as an indicator.

Analytical Process.—Place 100 cubic centimeters of the soil extract in a clean white casserole and concentrate to about 25 cubic centimeters. Add a few drops of potassium chromate and titrate drop by drop with the silver nitrate solution, stirring continuously. As each drop of silver nitrate is added, a reddish-brown precipitate of silver chromate will be formed. As this precipitate is decomposed by the alkali chloride, it will disappear on stirring.

$$Ag_2CrO_4 + 2NaCl = 2AgCl + Na_2CrO_4.$$

After the chlorine is all precipitated, the next drop precipitates silver chromate, which colors the solution a permanent reddish-brown. Each cubic centimeter of the standard silver nitrate required is equivalent to 0.5 milligram of chlorine.

Total chlorides may be determined more accurately by fusing 1 gram of soil with chlorine-free sodium carbonate or bicarbonate, cooling and disintegrating with hot water. When cold, the solution is treated with dilute nitric acid, filtered and the filtrate used for the precipitation with silver nitrate.[19]

Determination of Sulfates:

Reagents:

1. *Barium Chloride.*—Pure salt, powdered.
2. *Hydrochloric acid,* concentrated.
3. *Standard Potassium Sulfate Solution.*—Dissolve 0.9071 gram of pure potassium sulfate in water and dilute to 1,000 cubic centimeters. Each cubic centimeter of this solution contains 0.5 milligram of SO_4.
4. *Standard Turbidity Solution.*—Dilute 20 cubic centimeters of the standard sulfate solution to 1,000 cubic centimeters. Treat 25, 50, and 100 cubic centimeter portions as described under analytical process below and use as standard for comparing the turbidity of the solutions. If more dilute solutions are required, the smaller portions may be diluted to 100 or 200 cubic centimeters as the occasion demands. The concentration of the diluted solutions can be easily calculated.

Analytical Process.—Measure out 50 cubic centimeter portions of the solution to be tested and place in 200 cubic centimeter Erlenmyer flasks. Add 1 to 2 drops of concentrated hydrochloric acid and 0.1 to 0.2 gram of barium chloride, stopper tightly and shake thoroughly at intervals for 30 to 40 minutes. At the same time treat the standard solutions in the same manner. Compare the turbidity of the unknown solutions with that of the standards in the colorimeter and calculate the amount of sulfates in the original solution in the same manner as directed for the phenoldisulfonic acid method for nitrates. The photometric method may be used in place of the colorimetric. (See Sulfur, page 160.)

ALUMINUM

Aluminum is usually determined by means of the alkali fusion method, precipitating with iron, etc. (For the details see Com-

plete Soil Analysis, page 166.) In spite of the fact that it occurs rather abundantly in soils it has no effect on plant growth unless it is in the so-called "Active" state.

"Active" Aluminum.—"Active" aluminum[6] is that portion of the soil element that is capable of influencing or inhibiting the growth of plants. It is not soluble to any appreciable extent in the usual solvents for soil elements.

Determination of "Active" Aluminum:

Reagents:

1. $\frac{N}{2}$ acetic acid.
2. 50 per cent acetic acid.
3. Dilute (1 to 1) hydrochloric acid.
4. 10 per cent ammonium phosphate solution.
5. 15 per cent ammonium acetate solution.
6. $\frac{N}{1}$ sodium thiosulfate.

Analytical Process.—Place 100 grams of moist fresh soil (drying the sample increases its active aluminum content) in a 1,000-cubic centimeter shaker bottle, add 500 cubic centimeters of $\frac{N}{2}$ acetic acid and shake 1 hour. Let stand overnight and filter the supernatant liquid through a Pasteur-Chamberland filter. Pipette off 250 cubic centimeters, place in a beaker, add 10 cubic centimeters aqua regia and evaporate to dryness, first on the hot plate and then finish on the steam bath. Moisten the residue with 10 to 15 cubic centimeters of (1 to 1) hydrochloric acid and evaporate to dryness, twice, to dehydrate the silica. Digest the residue with 100 cubic centimeters of hot dilute hydrochloric acid and filter, washing the filter paper clear of chlorides with hot water. The filtrate should measure about 200 cubic centimeters. To the filtrate, add a solution of ammonium phosphate (5 cubic centimeters of a 10 per cent solution) in excess and bring to a boil. Add 2 cubic centimeters of $\frac{N}{1}$ sodium thiosulfate solution and continue boiling for at least 10 minutes to reduce completely any ferric iron that may be present. Exactly neutralize with ammonium hydroxide, avoiding any excess, and immediately acidify very slightly (a drop at a time) with 50 per cent acetic acid. Boil, let precipitate, subside, and filter. Wash the precipitate once

with hot water and dissolve the aluminum phosphate from the free sulfur on the filter with hot, dilute (1 to 6 HCl), refiltering if solution at first comes through turbid. Wash the filter with hot water until free of chlorides (about 200 cubic centimeters). Precipitate the aluminum phosphate by adding first, to the hot filtrate, 5 cubic centimeters of the 10 per cent ammonium-phosphate solution, then dilute ammonium hydroxide until a slight permanent precipitate just begins to form, then 10 cubic centimeters of a 15 per cent solution of ammonium acetate. The hot solution should test slightly acid to litmus at this point. Boil and filter as above, wash free from chlorides with hot water. (A third precipitation may be necessary to separate completely the calcium and aluminum phosphates where the soils employed have recently received large applications of either acid phosphate or lime.) Ignite the precipitated aluminum phosphate ($AlPO_4$) to constant weight, and calculate in parts per million.

AMMONIA

The formation of ammonia in manures or soils is due to microbiological activities, see "Biological Production of Ammonia" Section 4, page 188. The rate of formation in the soil is taken as an indication of the fertility of that soil. As ammonium compounds are not subject to excessive losses by leaching and are more or less rapidly oxidized to the available nitrates, they form one of the most valuable sources of nitrogenous fertilizers.

Ammonia is usually combined with some acid. It is freed from its combinations by the addition of an alkali and distilling, or aerating, catching the distillate in an excess of standard acid and titrating.

$$(NH_4)_2SO_4 + 2NaOH = 2NH_3 + Na_2SO_4 + 2H_2O$$

If in a fairly pure state, *i.e.*, chloride or sulfate, the ammonia may be readily determined directly in the original solution by the colorimetric process (Nesslerization).

Determination of Ammonium Salts:

(Fertilizers, etc.)

Reagents:

1. Heavy magnesium oxide.
2. Standard $\frac{N}{10}$ acid.
3. Standard $\frac{N}{10}$ alkali.

Analytical Process.—Weigh out 0.5 to 2 grams of the sample in duplicate according to the purity of the material and place in 500-cubic centimeters Kjeldahl flasks. Add 250 to 400 cubic centimeters distilled, ammonia-free water, a small piece of paraffine the size of a pea, and 8 to 10 grams of heavy magnesium oxide. Connect with the Kjeldahl distillation apparatus and distill over, 150 to 200 cubic centimeters into an excess of a carefully measured amount of $\frac{N}{10}$ acid. Titrate the excess of acid using alazarin red indicator and calculate the per cent of ammonia in the sample.

If the determination is to be made by the aeration method use a few drops of a heavy oil instead of paraffine and rub the MgO to a cream with water before adding. The material should be vigorously agitated by the air current for 4 hours.

Calculations.—It has been shown that 1 cubic centimeter of any $\frac{N}{10}$ acid is equivalent to 1.401 milligrams of nitrogen (see Calculation of the Equivalent Factor of Elements in Normal Solutions, page 59). Assume that 70 cubic centimeters of $\frac{N}{10}$ acid have been used in the receiving flask and that 14.5 cubic centimeters of $\frac{N}{10}$ alkali are required to titrate to the neutral point. By difference, we find that 55.5 cubic centimeters of the $\frac{N}{10}$ acid have been neutralized by the ammonia distilled over. We know that 1 cubic centimeter of $\frac{N}{10}$ acid is equivalent to 1.401 milligrams of nitrogen, then $55.5 \times 1.401 = 77.75$ milligrams of nitrogen. The nitrogen is converted to ammonia by multiplying by the factor 1.2143. In the case of microbiological determinations the results are expressed as milligrams nitrogen (mgs. N.) as ammonia.

In case the amount of ammonia given off is too small to measure accurately by titration, the distillate may be Nesslerized, and the ammonia determined colorimetrically. This process is frequently made use of in all determinations involving the measurement of small amounts of nitrogenous compounds which have been converted to ammonia.

Total Ammonia in Manures and Soils.—The direct determination of ammonia in manures and soils by the aid of heat at

the boiling temperature is questionable, due to the fact that some amino compounds are usually present and may decompose in the presence of alkalis. This danger is apparently overcome by the use of the aeration unit, (see Method 2).

Method 1 (Distillation)

Reagents:

Same as for ammonium salts.

Analytical Process.—Place 100 grams of soil, or 10 to 25 grams of manure, in a 500-cubic centimeter copper distilling flask, add 300 cubic centimeters of ammonia-free water, a small piece of paraffine, and 5 to 10 grams of heavy magnesium oxide. Connect with the Kjeldahl distilling apparatus and distill 150 to 200 cubic centimeters into standard acid. This determination must be continually watched to prevent boiling over. The boiling may be effectively checked by the application of cold, wet cloths directly to the flasks.

The distilled over ammonia is determined and calculated as in the determination of an ammonium salt.

Method 2 (Aeration)

Reagents:

Same as for ammonium salts, except that sodium carbonate is used instead of magnesium oxide.

Analytical Process.—Weigh out 100 grams of soil, or 5 to 20 grams of manure and place in 500-cubic centimeter Kjeldahl flasks. Add 300 cubic centimeters of ammonia-free distilled water, 2 to 3 cubic centimeters heavy oil, and 8 to 10 grams of sodium carbonate. Place the standard acid in the receiving flask (milk bottle) and add enough neutral distilled water to make approximately 250 cubic centimeters. Also add the indicator and adjust the bulb through which the ammonia-laden air is to pass as near to the bottom of the flask as possible. Adjust the long tube in the Kjeldahl as closely as possible to the point where the flask touches the table. Connect as a member of the aeration train and aerate vigorously for 4 to 5 hours. If a temperature of 75°C. is maintained,[14] by keeping the Kjeldahls in a trough of hot water, the time may be reduced to 1 to 1½ hours. The air should be passed through the apparatus rapidly enough to keep the soil or manure thoroughly agitated at all times.

At the end of the aeration period disconnect, wash the end of the tube that has been in the acid with neutral water, and titrate in the usual manner.

FREE AMMONIA IN SOILS AND MANURES

Method 1

Reagents:

1. Same as for ammonium salts.
2. Concentrated hydrochloric acid.

Analytical Process.—Place 100 grams of fresh soil, or 10 to 25 grams of manure, in 500-cubic centimeter shaker bottles, add 200 cubic centimeters of ammonia-free water and enough concentrated hydrochloric acid (10 cubic centimeters or more) to make the solution decidedly acid. Add enough water to make the total volume of the solution 400 cubic centimeters and shake 2 hours. Let settle several hours, decant through a dry filter, draw off an aliquot (100 to 200 cubic centimeters), place in a 500-cubic centimeter Kjeldahl flask, add sufficient ammonia-free water to make 250 cubic centimeters, a piece of paraffine, and 8 to 10 grams of heavy, magnesium oxide previously reduced to a cream. Distill and determine as in the case of ammonium salts.

Method 2

Useful in the determination of the biological production of ammonia as all action is stopped by the use of copper sulfate.

Reagents:

1. Ten per cent copper sulfate solution.
2. Concentrated sodium hydroxide solution.
3. Standard solution as for ammonium salts.

Analytical Process.—Transfer soil or manure to quart Mason jars using enough ammonia-free water to make the total volume of solution 490 cubic centimeters. Add 10 cubic centimeters copper sulfate solution. Shake every 20 minutes for 4 hours. Allow to stand overnight. Draw off 250-cubic centimeter portions and place in 500-cubic centimeter Kjeldahl flasks, add 10 cubic centimeters concentrated sodium hydroxide, a small piece of paraffine, and distill into standard acid in the usual manner.

Colorimetric Determination of Ammonia:

(Nesslerization)

Reagents:

Nessler's Solution: 1. Dissolve 50 grams of potassium iodide in a small quantity of cold distilled water, 150 to 200 cubic centimeters.

2. Add a saturated solution of mercuric chloride until a slight precipitate persists.

3. Add 400 cubic centimeters of a 50 per cent solution of a clear potassium hydroxide solution.

4. Dilute to 1,000 cubic centimeters, allow to settle for 1 week, decant, and keep in a well-stoppered bottle in the dark.

Ammonia-free Water. *Standard Ammonium Chloride Solution.*—Dissolve 3.82 grams of pure, dry, ammonium chloride in 1,000 cubic centimeters of distilled water. Dilute 10 cubic centimeters of this to 1,000 cubic centimeters with ammonia-free water. Each cubic centimeter of this dilution contains 0.01 milligram of nitrogen.

Test for Ammonia.—Add a drop of the above Nessler solution in a test plate to a drop of the solution to be tested. A deep golden-yellow color indicates the presence of ammonia.

Analytical Process.—Prepare a series of standards containing 0.01, 0.1, and 1.0 milligrams of nitrogen by placing the required amount of the standard ammonium chloride solution in a 100-cubic centimeter measuring flask, adding approximately 35 cubic centimeters of ammonia-free water, 2 to 5 cubic centimeters of the Nessler's solution, building up to the mark, without stirring, and allowing to stand for 10 minutes. Or, prepare a series of Nessler tubes containing 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, etc., as above, and dilute to 50 cubic centimeters.

Develop the color in the solution to be tested in the same manner and compare the color produced with that in the standards, using the colorimeter, as directed in the determination of nitrates, page 134. Calculate the results in the same manner as recommended for nitrates, page 136.

When the Nessler tubes are used, the depth of color is determined by looking vertically downward through the tubes, which are placed upon a white surface adjusted at an angle in order to reflect the light upward. The determination should be made in front of a window with a northern exposure. Calculate in the

same manner as directed in the colorimetric determination of nitrates.

BORON (BORIC ACID)

The determination of boron in fertilizers has recently become of great importance as small traces of this element seriously affect plant growth. It may occur in certain of the potash mixtures. The claim is made (Va. Agric. Exp. Sta. Rpt. 1918–19) that the determination of boric acid should be a regular laboratory procedure in the examination of fertilizers.

Determination of Boric Acid:

Boric acid does not readily form an insoluble salt but may be readily and completely separated from all other acids and bases* by distillation with methyl alcohol.[16]

Apparatus.—Bend the stem of a 150- or 200-cubic centimeter pipette close to the bulb at right angles to the axis, so that the whole will represent an U with sharp corners. One of the stems is cut off about 3 inches above the bend, the other (which should have an internal diameter of not less than 0.7 centimeter) is bent in the form of a gooseneck about 3 inches above the first right-angle bend. The short upright end is connected with a 50-cubic centimeter dropping funnel. The gooseneck is connected with an upright condenser, which in turn is fitted with a loose stopper, into a 250-cubic centimeter Erlenmyer flask. The bulb of the pipette is immersed in an oil or paraffine bath.

Reagents:

1. Dilute nitric acid free of chlorine or dilute acetic acid.
2. Methyl alcohol.
3. Calcium oxide freshly prepared and reduced to constant weight.

Analytical Process.—Fifty- to seventy-five cubic centimeter portions of the borate solution, which should not contain more than 0.2 gram of B_2O_3 are acidified slightly with dilute nitric or acetic acid, using phenolphthalein. One or two drops are added after the color disappears. Transfer the solution to the bulb of the pipette. The bulb is then made to serve as a retort by lowering gently into a paraffine bath heated to 130 to 140°C. At first only the bottom of the retort is allowed to touch the paraffine, but as the liquid distills off it is lowered until half of it is immersed

* Gooch method.

and all water is evaporated. Now add carefully, by means of the dropping funnel, 10 cubic centimeters of methyl alcohol and distill the solution to dryness. Repeat the addition of methyl alcohol and distillation to dryness at least six times. Test for any boric acid left in the retort by means of tumeric paper before proceeding with the determination.

The distillate is caught in a lime water solution prepared by placing exactly 1 gram of calcium oxide in the Erlenmyer flask and adding a little water.

After the sixth addition of methyl alcohol and subsequent evaporation to dryness, transfer the solution in the Erlenmyer flask to a tared platinum dish. Evaporate carefully to dryness. Ignite to change all calcium compounds to the oxide and weigh The increase in weight of the calcium oxide is due to B_2O_3.

Note.—In the case of soils, the insoluble boron compounds are brought into solution by fusion with an alkaline carbonate. If fluorine is present, it is removed as calcium fluoride in the aqueous solution of the fused materials. For fusion, see Complete Soil Analysis, Fusion with Sodium Carbonate, page 163.

Determination of Boric Acid in Organic Matter:

Reagents:

Lime water freshly prepared.

Analytical Process.—Weigh out 100 grams of the sample in a platinum dish, make strongly alkaline with lime water, heat cautiously until the organic matter is thoroughly charred and will give a colorless solution. Avoid overheating. Add 20 cubic centimeters of water and acidify with hydrochloric acid. Transfer the whole to a flask, add 0.5 gram of calcium chloride, a few drops of phenolphthalein and titrate with sodium hydroxide until a slight pink color remains, then add 25 cubic centimeters of lime water. Shake vigorously, filter through a dry filter and wash until the washings total 100 to 150 cubic centimeters. Use an aliquot of this solution for the determination of the boric acid by the Gooch method.

Boric acid may be determined by titration in a glycerine solution.[12]

CALCIUM

Calcium occurs relatively abundant in rocks, soil and plants. Its use from the fertility standpoint is mainly as a corrector of

soil acidity and less as a plant food. Its need is indicated by the acid reaction "Lime Requirement" of the soil. It is always more or less closely associated with magnesium, thus the determinations of the two elements go hand in hand. An accurate separation is difficult and is usually accomplished by bringing the elements into solution with hydrochloric acid and precipitating the calcium as the oxalate.

$$CaCO_3 + 2HCl = CaCl_2 + CO_2 + H_2O$$
$$CaCl_2 + (NH_4)_2C_2O_4 = CaC_2O_4 + 2NH_4Cl$$

The separation of the calcium and magnesium is accomplished in the hot solution, CaC_2O_4 being practically insoluble. In the case of carbonate rock analysis, great care must be taken due to the interference of strontium and barium, also to the preponderance of either of the main elements on the precipitation of the other. The interference of barium, and of an excess of either calcium or magnesium,[19] is usually removed by a second precipitation as directed below.

Determination of Calcium in a Limestone or Fertilizer:

Reagents:

1. Hydrochloric acid, 1 to 1 dilution.
2. Ammonium hydroxide.
3. Saturated ammonium oxalate solution $(NH_4)_2C_2O_4H_2O$ freshly prepared.

Analytical Process.—Place 1 gram of powdered, dry sample in a beaker, add about 10 cubic centimeters of water, cover the beaker with a cover-glass and introduce a long stirring rod in the lip of the beaker. Add slowly, by means of the stirring rod, about 20 cubic centimeters of 1 to 1 hydrochloric acid and digest carefully over a low flame until all carbon-dioxide is expelled. Wash any material that may have spattered on the cover-glass back into the beaker and filter out any undissolved residue. In calcium and magnesium determinations always use filters that have been acid washed as ordinary filters may contain traces of lime. Dilute the filtrate to 200 cubic centimeters, boil for at least 5 minutes, to expel all carbon-dioxide, make alkaline with ammonium hydroxide and again heat to boiling. Add to the hot solution 25 cubic centimeters of ammonium oxalate solution stirring constantly. Digest at 65 to 80°C. for about 4 hours to allow the precipitate to form and settle fully. Longer standing

results in a possible precipitation of the magnesium oxalate, while with a much shortened period, the calcium precipitation is liable to be incomplete. Decant the supernatant solution through a filter, keeping as much of the precipitate in the beaker as possible and washing by decantation, using about 20 cubic centimeters of hot water each time. As a small amount of magnesium may be carried down in the first precipitation it is recommended that the precipitate be dissolved in warm hydrochloric and reprecipitated a second or even a third time, proceeding as described above. The final precipitate will consist of calcium oxalate, and if any strontium is present, of strontium oxalate. (For the separation of strontium see below.)

Wash the calcium oxalate precipitate on the filter which was first used and wash with small successive portions of hot water until it is free of chlorides. The amount of calcium present may be determined gravimetrically or volumetrically.

Gravimetric Determination.—Dry the paper containing the chloride-free precipitate and ignite to constant weight in a tared crucible. Wash as calcium oxide. Do not leave in desiccator longer than 30 minutes as the CaO may make an appreciable gain in weight. For this reason, some workers prefer to determine the calcium as the sulfate. In this case, wet the CaO carefully with a slight excess of dilute sulfuric acid, dry and again ignite to constant weight, weighing as $CaSO_4$.

Volumetric Determination.—Dissolve the calcium oxalate in an excess of warm dilute sulfuric acid,

$$CaC_2O_4 + H_2SO_4 = CaSO_4 + H_2C_2O_4$$

and titrate with a $\frac{N}{10}$ potassium permanganate solution,

$$2KMnO_4 + H_2C_2O_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 4H_2O + CO_2.$$

Each cubic centimeter of $\frac{N}{10}$ $KMnO_4$ is equivalent to 0.0020 grams of calcium.

FACTORS

$$\text{Calcium} \times 1.3993 = CaO$$
$$\text{Calcium} \times 2.4974 = CaCO_3$$
$$\text{Calcium} \times 3.3975 = CaCO_4$$
$$\text{Calcium} \times 2.5810 = Ca_3PO_4$$

SEPARATION OF STRONTIUM *

Reagents:

1. Nitric acid.
2. *Ether-alcohol Mixture.*—Equal parts of absolute alcohol and ether.

Analytical Process.—Transfer the moist precipitate of calcium and strontium oxalate, wrapped in the filter paper, to a tared crucible, heat carefully to char without inflaming the paper. When the carbon disappears, increase the flame and heat for about 1 hour, or to constant weight. Place in desiccator, and weigh as soon as cool.

Transfer the weighed precipitate to a small flask, dissolve in nitric acid, and evaporate to dryness at about 150°C. Dissolve the calcium by adding ether-alcohol mixture, with gentle agitation, cork flask, and allow to stand overnight. Filter through the smallest possible filter and wash with the ether-alcohol mixture, allow filter to dry and dissolve out the strontium nitrate with a few cubic centimeters of hot water. A slight amount of residue may appear at this point, the weight of which is deducted from the original weight of the combined precipitation. To the filtrate containing the strontium nitrate add a few drops of sulfuric acid and an equal volume of alcohol, allow to stand for 12 hours, filter, ignite, and weigh as strontium sulfate, $SrSO_4$.

Determination of Calcium in Soil:

Reagents:

1. Pure sodium carbonate.
2. Hydrochloric acid, 1 to 1 dilution.
3. Approximately 10 per cent sodium carbonate solution.
4. Saturated solution sodium acetate.
5. Five per cent solution sodium acetate.

Analytical Process.—Make a fusion with sodium carbonate as directed under total soil analysis and bring it into an aqueous solution (see pages 163 to 165). Acidify the solution with hydrochloric acid in a 600-cubic centimeter beaker, keeping the volume to about 200 cubic centimeters. Add from a burette, with vigorous stirring, the 10 per cent sodium-carbonate solution until a slight opalescence is obtained. Test with litmus a drop of the solution, which should be distinctly but not strongly acid. Now add the equivalent of 2 or 3 grams of sodium acetate in the form

* *U. S. Geological Survey Bulletin* 700, pp. 142–145.

of a solution. Heat the solution to boiling and boil 1 minute; longer boiling will make the precipitate gelatinous. Allow the precipitate to subside and filter while hot through an acid washed filter, catching the filtrate in a 600-cubic centimeter beaker and washing several times with hot 5 per cent sodium-acetate solution. Reserve the filtrate. Transfer the filter with the precipitate to the original beaker and add just enough hydrochloric to dissolve. Macerate the filter paper, bring the volume to about 200 cubic centimeters and repeat the precipitation with sodium acetate as described above. If preferred, the precipitate may be dissolved on the filter but the paper aids in filtration. Wash the second precipitate of calcium acetate ten to fifteen times with hot, 5 per cent solution of sodium acetate. This precipitate may be dried, ignited, and weighed as CaO or combined with the calcium that is recovered in the filtrate.

As calcium acetate is slightly soluble, the combined filtrate will contain calcium, magnesium, and manganese. The latter must be removed in order not to affect the determination of the magnesium. After the manganese has been removed, separate the calcium and magnesium as described in the determination of calcium in limestone, using ammonium oxalate.

SEPARATION OF MANGANESE

Reagents:

Saturated solution of bromine water

Analytical Process.—Concentrate the filtrate, from the basic acetate separation, on the steam bath to 250 or 300 cubic centimeters. Add 10 cubic centimeters of bromine water and boil. The hydrated manganese will be deposited in a granular form. Continue the boiling until the volume has been reduced to approximately 100 cubic centimeters. Filter and wash seven to ten times with hot water.

The reason why sodium instead of ammonium salts is used in the determination of calcium in soils is because it is impossible to precipitate the manganese in the presence of the ammonium salts. The ammonium salts react with bromine according to the following:

$$2NH_4Cl + 3Br_2 = N_2 + 2HCl + 6HBr.$$

Two strong acids are formed which in turn react with the precipitated MnO_2 and redissolve it.*

*See, Treadwell & Hall (1919 ed.) page 124.

CARBON

Carbon is found in nature in both the organic and inorganic states. It is determined by oxidizing the organic form to carbon-dioxide or by freeing the inorganic carbonates, either by heat or by strong acids. Both of these forms may be roughly determined by the Loss on Ignition Method (see Sec. 2, page 40). The accurate determination of both forms of carbon requires the use of special apparatus. There is no method for the determination of organic carbon. The organic is determined by difference from the total and inorganic determinations.

The amount and kind of carbon in the soil is an indication of its fertility. Excessive amounts of carbonate carbon indicate an alkaline condition. Relatively large amounts of organic carbon usually indicate a fertile soil while excessive amounts indicates peats or mucks. The organic carbon of the soil is not capable of assimilation by higher plants. It serves as the source of food and energy for practically all microbiological activities, and is possibly the most important of all soil compounds influencing plant growth.

Determination of Total Carbon in Soils:

The only accurate means of determining the total carbon in soils is to burn the sample directly in a current of oxygen.[30, 2] The combined carbonates are driven off as carbon-dioxide by the heat while the carbonaceous compounds are also oxidized to the same form.

The Apparatus.—The apparatus is essentially the same as the Fleming combustion apparatus as used in the determination of carbon in iron and steel. The apparatus illustrated in Fig. 3 consists of the electrical furnace (1), equipped with a $\frac{7}{8} \times 24$-inch fused silica tube (2) in which the sample is placed. The heat of the furnace is controlled by a rheostat (3). The oxygen is released from a tank (4), passing through a scrubber (5), containing 30 per cent sodium or potassium hydroxide to take out any carbon-dioxide. A second scrubber (6) containing concentrated sulfuric acid serves to dry the gas. Complete drying and removal of the last traces of carbon-dioxide, is accomplished by passing the gas through a tower (7) containing a layer of fused calcium chloride overlaid with soda-lime. From this tower the purified gas passes through a mercury valve (8), which serves as an absorbent for any possible sulfur fumes and also assists in maintaining a

constant pressure. The entrance of the silica tube (2) is loosely plugged at the point where the tube enters the furnace with an asbestos plug made by rolling a cylinder of wire gauze 2 inches long, packing with fiber asbestos, and provided with a handle so that it may be easily withdrawn. This plug prevents the heat of the furnace affecting the rubber stopper at the point of entrance. The exit end of the tube is filled with a 5-inch core of coarsely granular cupric oxide, held in place by fiber-asbestos plugs. The position of this core should be such that the oxide is just even with, but not in, the exit end of the furnace. The cupric oxide serves to oxidize any carbon driven over, as carbon-monoxide.

After passing through the furnace, the gas is conducted into an empty safety bulb (9) arranged to catch any back suction from (10) which is a scrubber tube containing concentrated sul-

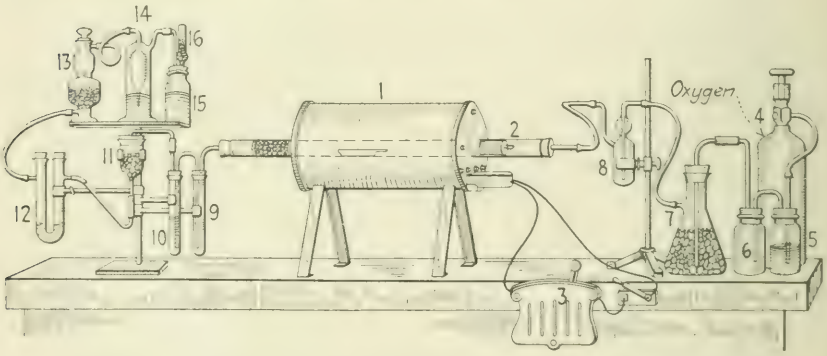

FIG. 3.—Apparatus for the determination of total carbon in soils.

furic acid, thus serving to remove any water and also preventing the packing of the granular zinc in (11). The tube (11) is an ordinary carbon funnel with a layer of glass wool in the bottom and filled with a 1 to 1½-inch layer of granular zinc (40 mesh). This zinc serves to remove any acid fumes, sulfur, chlorine, etc. After passing through the zinc the gas is thoroughly dried by passing through a U tube, the bottom of which is filled with glass wool and the arms filled with phosphorus anhydride. The gas containing the carbon-dioxide, freed of all interfering substances, is now passed into a tared Fleming bulb (13) where the carbon-dioxide is absorbed. This bulb is prepared for use by placing a layer of glass wool in the bottom and covering with a layer ¼ to ½ inch, of 20-mesh soda-lime, then filling the entire lower portion with 40-mesh soda-lime. The upper portion of the bulb is

filled with phosphorus-anhydride protected with glass wool. The phosphorus-anhydride insures the gas leaving the bulb with the same moisture content as on entering, thus eliminating error due to water gain or loss by the bulb.

The scrubber (14) containing concentrated sulfuric acid acts as a guard to the Fleming bulb and also as an indicator of the rate of gas flow. The bottle (15) contains barium hydroxide which serves as a telltale in case the absorption tower (13) should become exhausted. The barium hydroxide is protected by a soda-lime tube (16).

The complete apparatus, with modifications, may be purchased from any chemical supply house.

Analytical Process.—Carefully bring the furnace to a temperature of 900 to 950°C., about 1 hour preliminary heating will be required, and maintain this temperature throughout the determination.. Never heat the furnace over 1,000°C., as cupric oxide fuses at 1,064°C. When this occurs, the silica of the tube slags with the fused mass and the tube cracks on cooling. When the desired temperature has been obtained, place the Fleming absorption bulb in the train and adjust the glass plugs to allow the gases to flow through. Flush the apparatus with oxygen adjusting the flow so that 40 to 45 cubic centimeters will pass through it each minute. The rate of flow can be quickly determined by water displacement, at the same time counting the number of bubbles, in any of the scrubbers, that are required for each cubic centimeter. After the number of bubbles for each cubic centimeter is determined, the rate of flow may always be adjusted accordingly. Close the flow of gas, close the upper and the lower glass plugs in the Fleming bulb, remove the bulb from the train, and secure the open ends of the rubber tubing, either with pinch clamps or connecting with a piece of tubing. Determine accurately the weight of the bulb.

Weigh exactly 2 grams of dry soil (100 mesh or finer) and mix with approximately 2 or 3 grams of 40 mesh alundum. The addition of the alundum is to prevent fusion and sticking in the boats. Place the mixture evenly on the bottom of an alundum boat. Place the weighed Fleming bulb back in the train, being sure to adjust the glass plugs properly to allow a free passage of the gases. Remove the asbestos plug from the silica tube and insert the alundum boat into the tube by means of a long wire. The boat should be pushed to the center, or slightly beyond the

center, of the furnace. Quickly replace the asbestos plug, connect the apparatus, start and adjust the flow of gas, and allow to run for 20 minutes. At the end of the determination, disconnect the Fleming bulb and weigh carefully. The gain in weight is caused by the combustion of the organic matter and freed carbonates in the soil $CO_2 \times .2727 = C$.

REMARKS

To avoid error in the weighing of the Fleming bulb, a second bulb of approximately the same weight should be used as a counter balance. The large size of the bulbs causes them to be subject to changes in temperature, humidity, barometric pressure, etc.

One filling of the absorption bulb is sufficient for approximately 60 determinations.

With soils containing over 5 per cent total carbon, a 1-gram sample is sufficient.

After each combustion, it is advisable to tap the P_2O_5 tube to prevent the formation of channels.

Drawing the gas through by suction, or a combination of suction and pressure, is preferable to pressure alone due to the danger of leakage.

CARBONATE CARBON

The Determination of Carbonate Carbon.—(In the absence of carbohydrates.)

In the following determination of carbonate carbon, it is assumed that the sample does not contain any, or at least only a small amount of, carbohydrate material.

The inorganic or carbonate carbon may be determined by the use of the furnace as for total carbon above. Usually it is determined by one of the two following methods depending upon the amount of organic matters (carbohydrates) in the sample. The carbon is liberated directly from its base by the action of dilute hydrochloric acid.

$$CaCO_3 + 2HCl = CaCl_2 + CO_2 + H_2O$$

The evolved gas is measured in a gas burette, the carbon content being calculated from tables (see "Milligram of Carbon per Cubic Centimeter of Carbon Dioxide at Different Temperatures and Pressures" page 216), or it may be absorbed in alkali and measured by titration. (See Titration of Carbon Dioxide, page 119.)

The Apparatus.—The apparatus, illustrated in Fig. 4 is a modification of the Parr Carbon apparatus. The sample is placed in a 300-cubic centimeter Erlenmyer flask (3), which is fitted with a two-holed stopper. In one hole is inserted a separatory funnel (1), and in the other a tube (4), which conducts the evolved gas to the gas burette (7). The tube (4) should be cooled by a condensor (not shown) made of a piece of 1-inch

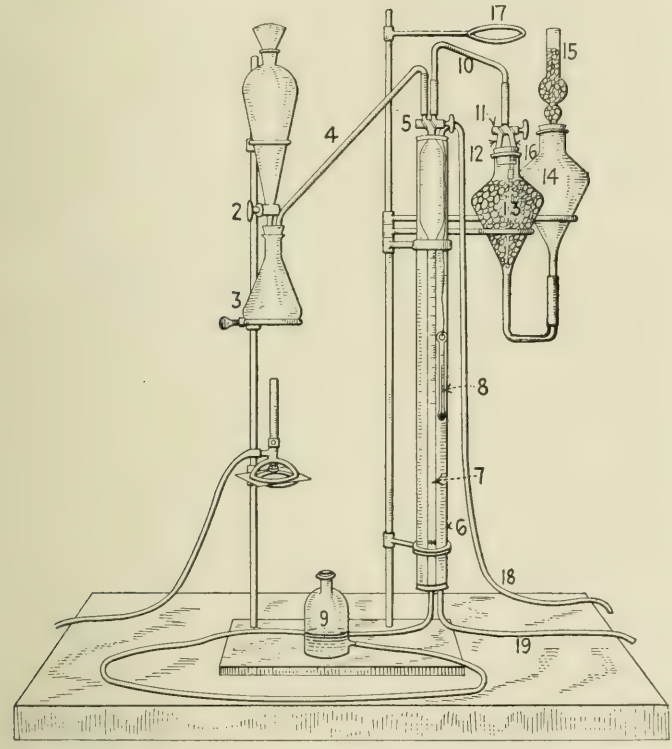

FIG. 4.—Apparatus for the determination of carbonate carbon in the absence of carbohydrates.

tubing 8 to 10 inches long. The gas burette (7) is completely enclosed in a large glass tube (6) secured at each end with stoppers and through which a stream of cool water, entering at (19) and overflowing through (18) continually passes. A thermometer (8) should be suspended in this water. The gas enters the burette through the double cock (5), displaces the solution, in the burette, which flows into the reservoir (9). The carbon-dioxide is absorbed in approximately 50 per cent saturation alkali (sodium or potas-

sium hydroxide) contained in the absorption bulbs (13) and (14). Bulb (13) is filled with short pieces of glass tubing or with glass beads, which assist in breaking up the bubbles, thus allowing complete absorption. The bulb (14) acts as a reservoir to take care of the overflow from (13) and is protected by a soda-lime tube (15). This bulb should be placed slightly higher than the absorption bulb in order to facilitate the fileing of the later.

Supplementary Apparatus.—Barometer for barometric readings.

Analytical Process.—Weigh out carefully 0.2 to 10 grams of the sample according to the character of the material. For limestones use 0.2 to 1.0 gram, for soils use 0.5 to 10.0 grams. Place the samples in 300-cubic centimeter Erlenmyer flasks. Cool the apparatus by running water through (19) until a constant temperature is obtained. Acidify, with hydrochloric, enough distilled water to fill the gas burette, and color strongly with methyl red indicator. Fill the burette by filling the reservoir (9), elevating to the position (17), and allow the air to escape through (4) by turning the cock (5). When the burette is full, close (5) and return (9) to the table as illustrated. Evacuate (13) by turning the cock (11) so that the air will flow through (12) into the gas burette. Allow the alkali solution to flow to a point in (12) midway between the stopper and the cock. Close (11) and mark this point. Now drive out all air from the gas burette, close (5). Add approximately 150 cubic centimeters of carbon-dioxide-free water to the sample in the Erlenmyer and connect with the apparatus. Close cock (2), open cock (5), so that the liquid in (7) will cause a slight vacuum in (3). At this point the liquid in (7) will fall slightly, but will come to a rest if there are no leaks. Pour 20 cubic centimeters of concentrated hydrochloric acid into (1) and introduce it slowly into (3) being careful not to admit any air. Fill (1) half-full with distilled water. Start the flame and bring contents of (3) to a boil, boiling briskly but carefully, for 2 or 3 minutes. If the tube (4) is not protected by a condenser, some of the steam may condense in the gas burette. This will not affect the determination, but some objectionable material may be carried over that will cloud the burette. Remove flame, carefully open (2), and allow the distilled water to drive out all air and gas from the flask and delivery tube into the burette. Close (5) as soon as the gas is driven over. Allow to stand so that the gas will be cooled to

a constant volume. Now bring the reservoir (9) to such a position that the height of the liquid in it will be level with that in the gas burette. Read this to the nearest 0.2 cubic centimeter.

Place (9) on (17), turn stopcock (11) to connect delivery tube (10) with the tube (16) which extends below the beads in the absorbent. Turn (5) to allow the gas from the burette to flow into the absorption bulb. As soon as the burette liquid rises into (10) turn (11) to communicate with tube (12), which opens at the top of the bulb (13), and allow the residual gas to pass back into the burette, taking care to stop the flow of alkali at the mark placed on (12). Read the burette as directed above, and repeat the absorption of the gas until a constant reading is obtained. The gas should be passed through the absorbent at least twice.

Observe the temperature of the water jacket as indicated by the thermometer (8) and the barometric pressure. From this data obtain from the table "Milligrams of Carbon per Cubic Centimeter of Carbon-dioxide at Different Temperatures and Pressures," page 216, the weight of carbon in each cubic centimeter of the absorbed gas. Subtract the second or constant reading from the first, the difference is the cubic centimeters of CO_2 absorbed. Calculate the amount of carbon as follows: cubic centimeters of CO_2 absorbed times the weight of carbon in each cubic centimeter of the gas at the observed temperature and pressure.

The Determination of Carbonate Carbon in the Presence of Carbohydrates.—Carbonate carbon cannot be determined accurately in the presence of carbonaceous material by the preceding method due to the fact that the strong acid decomposes some of the organic matter. The determination may be made by aspirating under reduced pressure. The apparatus and method described are modifications of those recommended by Truog. The carbon-dioxide is liberated under reduced pressure by dilute hydrochloric acid, absorbed in a known volume of standard alkali, precipitated from the solution and determined by the difference on titrating the excess alkali with standard acid.

Apparatus.—The apparatus illustrated in Fig. 5 consists of an evolution flask (1), a wash tower (8), and an absorption tower (11). The evolution flask is fitted with a dropping funnel (2),

with a stopcock (3) to admit acid to sample, and (4) is a two-way stopcock, one position of which connects the flask (1) with the entrance tube (24) which leads carbon-dioxide-free air from the purifying towers (5) and (5). The towers contain 40 per cent sodium hydroxide. The other position of (4) connects directly

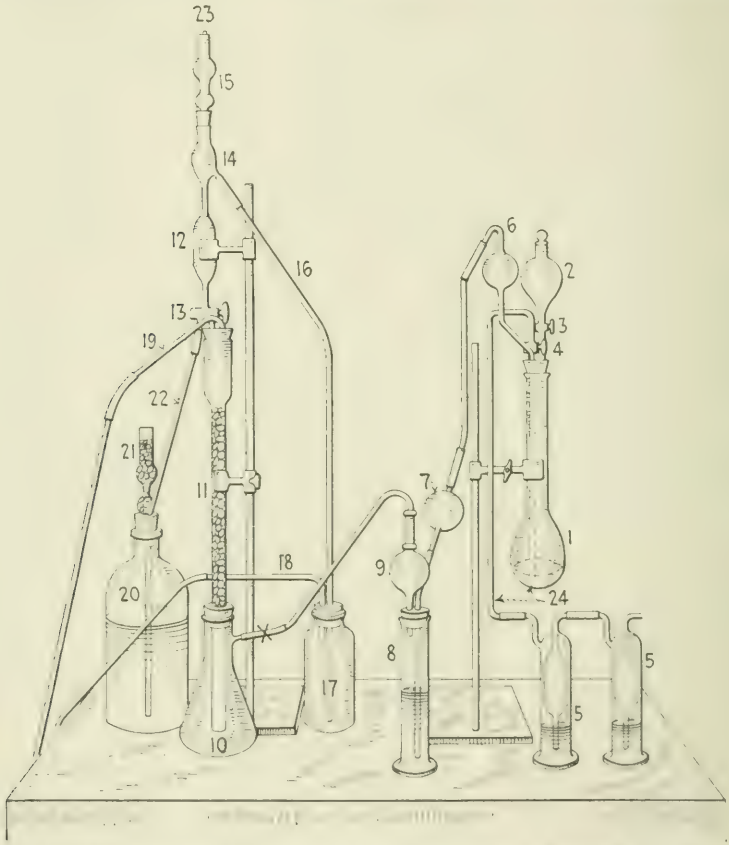

Fig. 5.—Apparatus for the determination of carbonate carbon in the presence of carbohydrates.

with the open air. The trap (6) acts as a condenser, preventing an excess of HCl or water vapor from passing over, while (7) is a trap to arrest inadvertent suck-back of the wash solution in (8). The wash tower (8) contains silver sulfate solution to remove any hydrochloric acid gas which may pass over.

$$Ag_2SO_4 + 2HCl = 2AgCl + H_2SO_4$$

Silver nitrate cannot be used in the tower because it would give rise to HNO_3 which is volatile.

The trap (9) is to prevent the solution in (8) from passing over. The connection is fitted with a stopcock (x) (not shown) to be turned off before releasing suction. The suction flask (10) forms a base for the absorption tower (11) which contains short lengths of glass tubing or beads, the diameter of which alternates from tier to tier, for breaking up the bubbles. This core is prevented from sliding out by a punctured, inverted glass thimble inserted in the lower end of the tube and held in position by a short, glass rod sealed across the bottom. Surmounting the core is a short, bulbed tube, the lower end of which fits loosely into the upper end of the narrow portion of the tower. The bulb serves to break up the last bubbles of gas and prevents splashing in the top of the tower. It also acts as an indicator in regulating the suction. The flask (10) should be raised about 2 inches and supported on blocks. The other essential parts of the apparatus are:

(12) automatic pipette for delivering a constant volume (50 cubic centimeters) of standard alkali to the tower,

(13) two-way stopcock on pipette for filling and discharging,

(14) overflow chamber,

(15) soda-lime tube to exclude CO_2,

(16) connection with overflow trap,

(17) overflow trap,

(18) connection with suction pump for operating pipette,

(19) connection with suction pump for aspirating,

(20) reservoir for absorbent,

(21) soda-lime tube to protect absorbent,

(22) supply tube leading to pipette,

(23) orifice to be stopped with finger tip while filling pipette,

(24) air inlet at bottom of evolution flask to insure agitation of sample.

Reagents:

1. Standard sodium hydroxide $\left(\text{approximately } \frac{N}{2}\right)$ for absorbent, prepared free from carbonates.

2. Standard acid—$\frac{N}{10}$ HCl—for titrating excess alkali.

3. Indicator—phenolphthalein, 1 gram in 100 cubic centimeters ethyl alcohol.

4. Dilute hydrochloric acid—1 to 5.
5. Barium chloride ($BaCl_2.2H_2O$) powdered crystals.
6. Distilled water free from carbon-dioxide.

Analytical Process.—Make sure apparatus is clean and in proper working order. Test tightness of stoppered joints by squirting water on them while suction is on. If in doubt as to tightness of joints and connections, run a blank determination.

Pulverize the soil or other material to pass a 100-mesh screen. Base the size of charge on a qualitative test: apply a few drops of dilute hydrochloric acid to a portion of the sample and note by the effervescence whether relatively large or small amounts of carbonate are present. Use 3 to 5 grams of materials high in carbonate, and correspondingly more, up to 50 grams, for those low in carbonate.

Introduce the charge into the evolution flask and add 30 cubic centimeters of CO_2 free water, rinsing down any particles adhering to the neck.

Fix the flask in position as shown, making sure that it connects tightly with the stopper and that the end of the inlet (24) touches the bottom of the flask lightly. The inlet tube is adjustable as to length, being connected below the stopper with rubber tubing.

See that stopcocks (3) and (13) are closed; then turn on water pump to give a suction of about 10 inches as shown by the mercury manometer, open stopcock (*x*), and turn stopcock (4) to admit CO_2 free air. Aspirate freely for 5 minutes to free the apparatus from CO_2. While this is being done place in funnel (2) 30 cubic centimeters of 1 to 5 hydrochloric acid, and fill the automatic pipette (12) with the absorbent.

Reduce suction slowly to 5 inches, then slowly admit the absorbent into the tower, allowing at the end, passage of a slight trace of air, which is CO_2 free, to flush the pipette tip.

Admit the 30 cubic centimeters of 1 to 5 HCl from funnel (2), taking care to prevent any air entering after it. It is best to leave about $\frac{1}{2}$ cubic centimeter of acid in the funnel, but to be sure to wash it out before the next determination. The concentration of acid in the flask is reduced to 1 to 10, which is ample for disintegrating calcium carbonate. If the presence of magnesium carbonate is known or suspected in the sample, it will be necessary to heat the flask gently so that the contents boil evenly under the reduced pressure for about 10 minutes. The

use of more concentrated acid is not desirable because of its tendency to liberate carbon-dioxide from the organic matter.

Adjust stopcock (4) carefully to admit just enough carbon-dioxide-free air so that the bubbles rising in the absorption tower cause the bulb above the core to ride gently while the suction is maintained at 5 inches. Too rapid flow of air may prevent complete absorption of the evolved carbon-dioxide and may cause loss by splashing in the top of the tower. A sluggish flow of air on the other hand, will not sufficiently agitate the sample in the evolution flask. Suction greater than 5 inches is avoided to safeguard against leakage; less than a 5-inch vacuum renders evolution of carbon-dioxide from the sample more difficult with dilute acid.

After aspirating 30 minutes close stopcock (*x*), turn (4) to communicate with the open air, and release suction. Remove blocks supporting (10) and disengage the absorption tower and its flask together. Have ready, in a narrow-mouth flask, about 950 cubic centimeters of carbon-dioxide-free distilled water containing 10 drops of phenophthalein. Use about 750 cubic centimeters of this to wash down the tower thoroughly. Remove tower from the flask and rinse off lower end, collecting all washings in the flask. Carefully transfer, through a funnel, the contents of the flask to a 1,000-cubic centimeter volumetric flask, washing thoroughly. Make volume to the mark with carbon-dioxide-free water and mix well; add 1 to 5 grams of powdered barium chloride crystals until precipitation is complete. Withdraw 100-cubic centimeter aliquots and titrate with $\frac{N}{10}$ HCl. From the data obtained calculate the per cent of carbon-dioxide in sample.

REMARKS

1. The standard alkali used as absorbent must be free from carbonate to prevent introduction of a plus error. While carbonate, originally present, would be included in the standardization, it would be thrown from the solution with that absorbed at the end of a determination; hence the titration would be lowered: In this connection the following facts should be borne in mind.

(*a*) Sodium carbonate hydrolyzes to give an alkaline reaction and hence shifts the end point in titration.

(*b*) Sodium carbonate renders the end point less sharp and positive Local concentrations of the mineral acid at the surface

of the solution, during the titration, are likely to decompose the carbonate with a loss of carbon-dioxide. This could be avoided by vigorous shaking and slow addition of the acid, but an opposite error would be induced by the more favorable opportunity for the solution to absorb carbon-dioxide from the air.

2. The absorbent may be prepared free from carbonate in the following manner: Quickly wash in distilled water, approximately 100 grams of sodium hydroxide to remove the coating of carbonate; then dissolve it in $2\frac{1}{2}$ liters of distilled water and add 5 grams of powdered $BaCl_2$ crystals to precipitate any carbonate in solution. Filter the solution through an extracted, asbestos pad on a Buchner funnel, directly into the reservoir supplying the automatic pipette, using a light suction. During this filtration the pad should always be kept covered with the liquid to prevent entrance of carbon-dioxide into the reservoir. It is also desirable to prepare a small excess of the alkali so that the reservoir may be filled nearly to the stopper before releasing suction. Where a large number of determinations are to be made, a correspondingly larger reservoir should be used to permit making up absorbent in large batches.

3. For accuracy and uniformity between checks it is necessary to observe the following:

(*a*) Use calibrated volumetric apparatus.

(*b*) In standardizing the absorbent use 50-cubic centimeter portions as delivered by the automatic pipette.

(*c*) Standardize the absorbent at average room temperature. This requires that it be allowed to stand several hours after preparation to permit dissipation of the heat of solution.

(*d*) Use a rapid and uniform procedure in transferring the absorbent and marking up to volume, and use only a gentle rotary shaking in titrating the aliquots. In this way a negligible error due to absorption of atmospheric carbon-dioxide will be involved.

(*e*) Always use the same amount of indicator.

(*f*) Use only carbon-dioxide-free distilled water. This may be prepared from ordinary distilled water either by boiling 15 minutes and allowing it to cool under a soda-lime tube, or by aerating with carbon-dioxide-free air for 24 hours.

4. The double titration method for carbonates could be used and the precipitation with barium chloride eliminated, but this method is open to objections cited under *a* in 3 above.

5. In the original method barium hydroxide was used as absorbent and a loose core of heads which slid into the flask at the close of a determination was used in the tower. Titration was then made directly in the flask.*

The Titration of Carbon-dioxide.—The absorption of carbon-dioxide is accomplished in an excess of alkali and therefore the determination must be made in a mixture of the free alkali and the basic carbonates. Phenolphthalein is added as an indicator and the excess alkali is approximately neutralized with a strong acid solution $\frac{2N}{1}$ or $\frac{N}{1}$, until an indication is shown that the neutral point is being approached, when $\frac{N}{10}$ acid is used to complete the neutralization. At this point, the basic carbonates are changed to the acid or bicarbonates.

$$NaOH + Na_2CO_3 + 2HCl = 2NaCl + NaHCO_3 + H_2O.$$

Due to the change in ionization and the liberation of (HCO_3) ions the pink color of phenolphthalein disappears. No attention is paid to the amount of acid required to reach this point but extreme care must be exercised to prevent overtitration.

Two drops of methyl orange per 100 cubic centimeters of solution are now added. Too much indicator tends to mask the results and make indefinite the very faint change when the neutral point is reached. Make careful note of the reading of the $\frac{N}{10}$ acid burette, use a white background to detect the faint color change, and compare the color with that of a solution containing the same amount of indicator and known to be neutral. Titrate to the neutral point, thus completing the reaction,

$$NaHCO_3 + HCl = NaCl + H_2CO_3.$$

Carefully read the number of cubic centimeters of $\frac{N}{10}$ acid required to complete the reaction and calculate the amount of CO_2 in the original solution as follows: Each cubic centimeter of $\frac{N}{10}$ acid is equivalent to 4.4 milligrams of CO_2.

It is important that the acid used in both titrations be introduced under the surface of the solution to be titrated in order

* For further discussion of the various points involved, consult the following: *Jour. Ind. and Eng. Chem.*, **7**; 227, 1045. 1915; 8: 341. 1916.

to avoid any loss. This may be accomplished by attaching a glass tube to the delivery tube of the burette.

A more pronounced color change in this titration[31] is secured by substituting thymol blue for phenolphthalein and bromphenol blue for methyl orange. Eight drops of thymol blue are added to each 100 cubic centimeters of solution. This gives a deep blue which changes to a muddy green when the hydrate is all neutralized and the carbonate converted to the bicarbonate. A slight excess of acid gives a lemon color which may again be brought to the neutral point. The addition of four drops of bromphenol blue will change the solution to a deep blue which continues until all the carbonates have been destroyed, when the same intermediate change of a muddy green for neutrality, and a lemon yellow for acidity, becomes apparent.

As the titration of carbon-dioxide is difficult under the best of conditions, due to the indefinite end point, it has been suggested that more accurate results would be secured if the alkali solution, containing the carbonates, were treated in the Parr apparatus, just the same as though it were a limestone.

CYANIDES

The nitrogen in fertilizers may be supplied in the form of calcium cyanamide $Ca(CN)_2$. After storing for various periods certain changes take place in this material. The cyanamide becomes mixed with calcium hydroxide, calcium carbonate, dicyandiamide, urea, and possibly other nitrogen compounds.

$$Ca(CN)_2 + 2H_2O = Ca(OH)_2 + CN_2H_2$$
$$CN_2H_2 + H_2O = CO(NH_2)_2.$$

Detection of Cyanides.—Shake approximately 5 grams of the material to be tested with about 100 cubic centimeters of distilled water and filter. Place 10 cubic centimeters of the clear solution in a test tube, make alkaline with dilute sodium hydroxide, add a few drops of 10 per cent ferrous hydroxide solution, and gently warm to convert the CN into ferrocyanide. Acidify the solution with hydrochloric acid and add a few drops of 10 per cent ferric chloride solution. A deep blue precipitate, "Prussian Blue" indicates cyanides.

ESTIMATION OF CYANIDES

The estimation of the various cyanide compounds above, depends on the fact that cyanamide is precipitated by a salt of

silver, in the presence of an excess of silver, while the silver dicyandiamide is soluble but is precipitated by the addition of potassium hydroxide.[40]

Determination of Cyanamide Nitrogen:

Reagents:

Ammoniacal Silver Solution.—One hundred grams of silver nitrate are dissolved in distilled water and made up to 1,000 cubic centimeters. Add 400 cubic centimeters of 10 per cent ammonium hydroxide.

Analytical Process.—Weigh out 10 grams of the material and place in a shaker bottle, add 500 cubic centimeters of distilled water, and shake for 2½ hours. Filter through a dry filter. Pipette off a 250-cubic centimeter aliquot and treat with the ammoniacal silver solution until no more precipitate is formed. Dilute to approximately 400 cubic centimeters by the addition of distilled water, filter through a dry filter, preferably by decantation, saving the filtrate for the determination of dicyandiamide nitrogen and urea. Wash the precipitate, discard the washings, and determine the nitrogen in the precipitate by the regular Kjeldahl method for total nitrogen.

Be sure that the filtrate is not allowed to become dry by standing around the laboratory and that all washings are disposed of at once.

Determination of Dicyandiamide Nitrogen:

Reagents:

Saturated solution of potassium hydroxide.

Analytical Process.—To 300 cubic centimeters of the filtrate from the cyanamide determination, add an excess of potassium hydroxide solution, dilute to 400 cubic centimeters, bring to a boil, cool, filter, wash, and determine the nitrogen in the precipitate by the regular Kjeldahl method. Reserve 300 cubic centimeters of the first filtrate for the determination of urea.

Determination of Urea Nitrogen:

Analytical Process.—Precipitate all excess silver in 300 cubic centimeters of the filtrate from the determination of dicyandiamide nitrogen with hydrogen sulfide, remove the hydrogen sulfide with a strong current of carbon-dioxide. Dilute to 400 cubic centimeters, allow to settle, and determine the urea in an aliquot after evaporating to dryness.

LIMESTONE

The agricultural value of limestone depends upon its fineness of division and its purity. The fineness of division is determined by sieves as in "The Mechanical Analysis of Soils," Section 1, page 15.

The purity of limestone is based on the fact that the effective calcium and magnesium are in the form of carbonates and as such are readily acted upon by dilute acids with the evolution of carbon-dioxide. As the carbon-dioxide will pass off as a gas, thus causing a loss in weight of the sample, advantage has been taken of this fact to place on the market several types of so-called, "Lime Testers." These testers are accurate enough for all practical purposes. For analytical determinations see Calcium and Magnesium.

Determination of the Purity of Limestone:

Method No. 1

Determine by the modified Parr apparatus, (see Carbonate Carbon, page 110).

Method No. 2

A sometimes quicker test is based on the fact that the base in the sample will combine with, and neutralize, an acid. If an excess of a known amount of standard acid is used the excess may be titrated.

Reagents:

$\frac{N}{2}$ hydrochloric acid.

$\frac{N}{10}$ alkali.

Analytical Process.—Weigh out accurately 0.2 to 1.0 gram of the sample according to the amount of carbonate it is expected to contain. Add 30 cubic centimeters $\frac{N}{2}$ hydrochloric acid, and approximately 100 cubic centimeters of distilled water. Bring to a boil for 2 to 3 minutes. Add a few drops of phenolphthalein and titrate the excess with $\frac{N}{10}$ alkali in the boiling solution, or add bromphenol blue and titrate in the cold. If the sample is not

readily attacked by the acid connect a reflux condenser to the flask and boil 1 to 2 hours, titrating as above.

Calculations.—Each cubic centimeter of $\frac{N}{2}$ hydrochloric acid neutralized is equivalent to 22 milligrams of carbon-dioxide.

BURNED LIME

Limestone is frequently burned to make its action quicker,

$$\begin{matrix} CaCO_3 \\ \\ MgCO_3 \end{matrix} + \text{heat} = \begin{matrix} CaO \\ \\ MgO \end{matrix} + CO_2.$$

This material forms an important source of agricultural lime. The oxides quickly absorb the moisture and carbon-dioxide of the atmosphere,

$$CaO + H_2O = Ca(OH)_2$$
$$Ca(OH)_2 + CO_2 = CaCO_3 + H_2O.$$

Thus, the commercial sample is a mixture of the oxides, hydroxides, and carbonates.

Determination of a Burned Lime:

Determine the carbonates as indicated for the limestone samples, using the modified Parr apparatus.

Determination of Hydroxides and Oxides:

Reagents:

Ten per cent barium chloride solution.

Analytical Process.—Place 1 gram of the sample, previously dried overnight at 110°C., in a 500-cubic centimeter measuring flask, fill the flask about two-thirds full with hot water, and shake thoroughly to convert all the oxides to hydroxides. Cork tightly, allow to cool, make up to the mark, shake, and allow to settle. Pipette off 100 cubic centimeters into a 250-cubic centimeter Erlenmyer flask. Any carbonate that is present is precipitated by the addition of a few cubic centimeters of 10 per cent barium chloride. Add a few drops of phenolphthalein and titrate slowly with $\frac{N}{10}$ hydrochloric acid. Calculate as directed under Limestone.

This result gives both the oxides and the hydroxides, calculate the two by difference as follows: Ignite a weighed amount of the original dried sample and weigh as the oxide. The carbonates as

well as the hydroxides will be converted to the oxide, while the oxides will remain unchanged. Knowing the weight of the oxide that will be formed from the carbonates and the weight of the mixed oxides and hydroxides, the hydroxides may be considered as the only thing that is changed, hence may be easily calculated.

MAGNESIUM

As magnesium is found always closely associated with calcium and as the calcium must be removed first, the determination given below assumes that the magnesium is in the filtrate from the previous manipulations. (See Determination of Calcium, pages 102 to 106.)

Magnesium seldom, if ever, occurs in the soil in a state detrimental plant growth. It is assumed to be more or less detrimental if it is applied to the soil in the form of high magnesic limestones.

Determination of Magnesium in a Limestone or a Soil:

Reagents:

1. Hydrochloric acid.
2. Ammonium hydroxide (specific gravity 0.96).
3. *Sodium Ammonium Hydrogen Phosphate Solution.*—One hundred grams of sodium ammonium hydrogen phosphate ($Na(NH_4)HPO_4 . 4H_2O$) is dissolved in 1,000 cubic centimeters of distilled water.
4. Two and one-half per cent ammonia solution.

Analytical Process.—Concentrate the filtrate from the calcium determination to about 200 cubic centimeters, cool, exactly neutralize with ammonium hydroxide and add, drop by drop, 10 to 15 cubic centimeters of the sodium ammonium hydrogen phosphate solution, stirring vigorously. This will precipitate almost all of the magnesium as magnesium ammonium phosphate, $MgNH_4PO_4$. The balance is precipitated by adding slowly to the solution one-third its volume of ammonium hydroxide, continuing the stirring. Never add the ammonium hydroxide first. The efficiency of the determination depends upon the thoroughness of the stirring. If the precipitation is carried out in 500-cubic centimeter Erlenmyer flasks and the solution thoroughly aspirated, preferably overnight, better results are secured. If several determinations are to be run, the flasks may be connected in series as members of an aeration unit.

The end or guard flask contains a solution of ammonia to replenish any loss. Any crystals that form on the aspirating tubes may be dissolved with warm hydrochloric acid.

Reduce the concentration of ammonium salts to a minimum by decanting the solution on a filter and dissolving the precipitate on the filter by washing with small quantities of warm hydrochloric acid and receiving the filtrate in the beaker containing the precipitate. Dilute to about 200 cubic centimeters, neutralize with ammonia, and reprecipitate as above omitting the aspiration. After decantation and washing with dilute ammonia solution, transfer the precipitate to the filter and wash free of chlorides with the same solution. Dry the precipitate, transfer the bulk to a glazed paper, ignite the filter paper in a tared crucible, add the precipitate and again ignite to constant weight. Weigh as magnesium pyrophosphate ($Mg_2P_2O_7$).

MANGANESE

Manganese seldom occurs in rocks in an amount exceeding 0.3 per cent. The United States Geological Survey[19] holds that the high figure commonly reported is due to analytical error.

The presence of soluble manganese in soils is claimed to be one of the factors inhibiting plant growth.

Detection of Soluble Manganese in Soils.[7]—Place 50 grams of air-dry soil (sieved 20 mesh) in a 500-cubic centimeter shaker bottle. Add 100 cubic centimeters of 5 per cent solution of potassium thiocyanate in methyl or ethyl alcohol and shake for 1 hour. If the solution is red, due to the presence of iron, add enough alkali to cause the red color to disappear, an excess of alkali may be used. If the solution develops a green color at any stage of the process it indicates the presence of soluble manganese in the manganic form.

Determination of Manganese:

(U. S. Bureau of Soils method)

Reagents:

1. Hydrofluoric acid.
2. Concentrated nitric acid.
3. Sulfurous acid (H_2SO_3).
4. One gram silver nitrate dissolved in 1,000 cubic centimeters of distilled water.

5. Ammonium persulfate.
6. Standard potassium permanganate solution.

Analytical Process.—Ignite 1 gram of soil in a platinum dish and digest overnight with 10 cubic centimeters of hydrofluoric acid and 1 cubic centimeter of concentrated nitric acid. Heat the residue until fumes of sulfur-trioxide are freely evolved, cool, cautiously add 10 to 15 cubic centimeters of distilled water and repeat the evaporation to dryness. Digest the residue with 50 to 65 cubic centimeters of distilled water and a few drops of sulfurous acid, to dissolve the manganese dioxide. Add the equivalent of 0.004 gram of silver nitrate, in solution, for each milligram of manganese that is present. Ordinarily, 0.005 gram of silver nitrate will be sufficient, but more is frequently needed, see below.

Heat the mixture over a low flame until the boiling temperature is reached, and filter into a 100-cubic centimeter measuring flask. The paper, funnel, and flask used for filtering should be washed thoroughly with hot water, before using, to remove any possible chlorides.

Add 1 gram of ammonium persulfate to the filtrate and heat on the steam bath until the permanganate color is fully developed.

Cool the solution, make up to a standard volume and compare the color produced with that of a standard manganate solution, similarly treated, using the colorimeter for comparison.

If a brown discoloration appears during the warming and after the addition of the silver nitrate, too little of the silver nitrate solution has been used. In such a case, reduce the solution with SO_2, add more silver nitrate, and proceed as above.

The standard (diluted) permanganate solution must be freshly prepared for each determination and there must be no loss of time in making the color comparisons. The dilute solution is made from the standard $\frac{N}{10}$ potassium permanganate solution.

In making a large number of determinations it is recommended that the method of Willard and Greathouse[41] be used.

NITROGEN

The amount of nitrogen in the soil is closely correlated with its crop producing powers. The relation of the total nitrogen content to the organic carbon content of the soil, the "Nitrogen – Carbon Ratio" is frequently taken as an index of soil fertility.

The determination of nitrogen is one of the easiest, and at the same time one of the most accurate, of all the analytical soil processes. The method used in soil analysis is that of Kjeldahl, or some modification of it. The process consists of completely decomposing the organic matter, by boiling in concentrated sulfuric acid. In the process the carbonaceous matter is oxidized and the nitrogen is converted into ammonia, which immediately forms ammonium sulfate. As the reaction between sulfuric acid and organic matter is slow, the oxidative process is hastened by the addition of a catalytic agent—mercury, copper, etc. The following is suggestive of the reactions involved:

Digestion:

$$2C_6H_4OHNH_2 + 27H_2SO_4 = (NH_4)_2SO_4 + 12CO_2 + 26H_2SO_4 + 4H_2O$$
$$(NH_4)_2SO_4 + HgSO_4 = Hg(NH_3)_2SO_4 + H_2SO_4.$$

Distillation:

$$Hg(NH_3)_2SO_4 + K_2S + NaOH = HgS + K_2SO_4 + 2NH_3 + NaOH$$

As the nitrate nitrogen is driven off as a gas when digested with sulfuric acid,

$$2NaNO_3 + H_2SO_4 = Na_2SO_4 + 2HNO_3$$

special methods must be used to reduce the nitrate radical to ammonia. In the case of soils, the amount of nitrate nitrogen is so small that, for all practical purposes, the ordinary Kjeldahl method suffices. Manures and fertilizers, however, usually contain large amounts of nitrate nitrogen.

Determination of Total Nitrogen.—(Exclusive of nitrate nitrogen.)

Kjeldahl Method

Reagents:

1. Concentrated sulfuric acid.
2. Metallic mercury.
3. Potassium permanganate crystals.
4. Concentrated sodium hydroxide solution.
5. *Potassium Sulfide Solution.*—Forty grams of K_2S dissolved in 1,000 cubic centimeters of water. For convenience the potassium sulfide solution and the sodium hydroxide solution may be mixed, usually at the rate of 1 pound of potassium sulfide to 80 pounds of sodium hydroxide.
6. Standard acid solution, usually $\frac{N}{7}$ sulfuric.

7. Standard alkali solution, usually $\frac{N}{14}$ sodium hydroxide.

8. Granular zinc, 40 mesh.

Analytical Process.—Weigh out samples as follows:

Soil	7 to 14 grams
Peat and muck	3 to 10 grams
Manures	2 to 5 grams
Plants, woody portions	5 to 10 grams
Plants, seeds	1 to 5 grams
Fertilizers, packing-house products	0.5 to 2 grams

Digestion.—Wrap the sample in filter paper in order to supply a liberal amount of carbon. This procedure is not necessary in the case of material containing large amounts of carbon. Place the sample in a dry 500 or 800-cubic centimeter Kjeldahl flask, add 20 to 30 cubic centimeters of concentrated sulfuric acid, and approximately 0.7 gram of mercury. The mercury is most conveniently added by means of a glass tube drawn out to a point and the point turned back so as to form a slight hook. The size of the opening should be so adjusted that 2 drops will be the right amount delivered. The sulfuric acid is poured carefully down the sides of the flask in order to wash down any adhering particles.

Place the flask in the digestion apparatus and heat slowly until all frothing ceases. Increase the flame and digest until colorless, 3 to 4 hours. Turn out flame and add immediately a few crystals of potassium permanganate, to oxidize any remaining organic matter. Add the crystals until the green color is permanent. Allow the flask to remain on the digestion rack until no more fumes are given off, remove, and allow to cool. If distillation is not to be made at once, tightly cork the flasks.

Distillation.—When the flasks are thoroughly cooled, and not before, add 200 cubic centimeters of ammonia-free water, 25 cubic centimeters of potassium sulfide solution, a piece of paraffine the size of a pea, and a sufficient quantity of concentrated sodium hydroxide to make the solution strongly alkaline. Add the hydroxide carefully by pouring down the sides of the flask in such a manner that it will form a layer in the bottom of the flask. Do not mix the two solutions at this stage as the ammonia is liberated in an alkaline solution and will be lost. The amount of hydroxide

to be used must be determined experimentally, usually 2½ cubic centimeters of a saturated hydroxide solution will be required for each cubic centimeter of concentrated acid used in the digestion. Add ¼ to ½ gram of granular zinc to prevent bumping. The zinc is quickly destroyed in the strong acid solution. It must never be added before the alkali. Connect the Kjeldahl flask immediately with the distilling apparatus, be careful not to mix the two solutions until all connections are tight.

Before neutralizing the acid, prepare the flasks for receiving the distillate by measuring out, into 500-cubic centimeter Erlenmyer flasks or pint milk bottles, sufficient standard acid to neutralize any ammonia that may be distilled over, using 5 to 10 cubic centimeters in excess. Place the flasks under the delivery tubes of the distilling apparatus. The flasks should be adjusted at such a height that the end of the delivery tube will be under the surface of the standard acid, in the receiving flask, at the beginning of the determination. A small block of wood, approximately 2 inches thick, is very good for this purpose. When the first rush of ammonia is over and the distilled water begins to collect in the receiving flask, the blocks should be removed to prevent any possible sucking back of the distillate.

When all connections are tight and the end of the delivery tube is under the surface of the standard acid, *carefully and very gently* rotate the Kjeldahl flask in order to mix the two solutions. Take plenty of time, as a mixture is being made of a strong acid and a strong alkali, considerable heat will be generated, and if the mixing is done too quickly the solution will boil over with great violence. When the mixture is complete, turn on the flame and heat gently until the solution boils and danger of frothing over is past. Distill over, into the standard acid, 125 to 150 cubic centimeters or more according to the amount of nitrogen in the sample.

Titrate the excess acid, using alazarin or methyl red as indicator, the same as for the determination of ammonia. Calculate in the same manner as directed for ammonia.

Students are frequently troubled with sulfur compounds distilling over and clouding the solution in the receiving flask. This is due to an insufficient amount of alkali, although enough may have been used to drive off the ammonia. As it is impossible to secure a reading with either of the above indicators in the presence of sulfur, the use of cochineal is suggested.

Aeration.—To the thoroughly cooled acid add 200 cubic centimeters ammonia-free water and 2 to 3 cubic centimeters of a heavy oil. Connect as a member of the aeration train and neutralize by sucking the alkali, previously mixed with the potassium sulfide solution, into the free end of the tube that extends to the bottom of the flask. The amount of alkali entering at any one time may be easily controlled by allowing a small amount of air to pass through the tube at the same time. Should the contents of the flask become too hot, a slight aeration will cool it. Wash out the tube with distilled water and aerate for 3 hours. In the case of the modified Kjeldahl determination the copper will serve as an indicator for the proper amount of alkali to be added. For preparation of aeration apparatus, see Aeration Unit, page 53.

Determination of Total Nitrogen.—(Exclusive of nitrate nitrogen.)

(A) Modified Kjeldahl Method

The procedures in the modified Kjeldahl methods are essentially the same as in the Kjeldahl except that some other metal is used instead of mercury. There is no objection to the use of mercury other than that potassium sulfide must be used to precipitate it in the distillation process. The following modifications are recommended:

(*a*) Five to ten grams of potassium sulfate K_2SO_4 and a small piece of copper wire (or 2 cubic centimeters of a 10 per cent copper sulfate solution) are used in each flask in place of the mercury.

(*b*) Ten to twelve grams of the following mixture is used in place of the mercury; potassium sulfate 100 grams, copper sulfate 5 grams and ferric sulfate 0.5 gram. Digest 2 hours after clearing.

(*c*) The ferric sulfate in (*b*) above is omitted and 5 to 8 grams of the potassium-copper sulfate mixture used.

The use of the above modifications is suggested when the amount of nitrogen in the sample is very small or when a long digestion period is required.

Determination of Total Nitrogen.—(To include nitrate nitrogen.)

In the analysis of compounds containing nitrate nitrogen some compound, usually salicylic acid, is added with the sulfuric acid to reduce the nitrates. The probable reactions are as follows:

Salicylic acid + sodium nitrate + sulfuric acid = nitrophenol, etc.

$$2C_6H_4{}^{OH}_{COOH} + 2NaNO_3 + H_2SO_4 = 2C_6H_4{}^{OH}_{NO_2} + 2CO_2 + Na_2SO_4 + 2H_2O$$

The nitrophenol is changed to aminophenol by nascent hydrogen formed by the action of acid on the zinc.

$$C_6H_4{}^{OH}_{NO_2} + 3H_2 = C_6H_4{}^{OH}_{NH_2} + 2H_2O.$$

The nitrogen in the aminophenol is changed to ammonium sulfate in the same manner as shown in the digestion process, page 127.

(B) Kjeldahl Method

Reagents:

The reagents are the same as in the regular Kjeldahl with the exception that each 30 cubic centimeters of the concentrated sulfuric acid contains 2 grams of salicylic acid and zinc dust is used.

Analytical Process.—Prepare the sample as directed for total nitrogen and place in 500- or 800-cubic centimeter Kjeldahl flasks. Add 30 cubic centimeters of sulfuric acid containing 2 grams of salicylic acid. Thoroughly mix with the sample, add 2 grams of zinc dust, and rotate the flask while adding. Digest until the white fumes disappear then add approximately 0.7 gram of mercury. Complete the determination as directed in the Kjeldahl method.

Determination of Total Nitrogen in a Mixture of Ammonium and Nitrate Salts

The nitrogen in fertilizers is frequently supplied in the form of ammonium and nitrate salts. The following method will determine only these two forms:

Modified Ulsch Method

Reagents:

1. Reduced iron.
2. Sulfuric acid, 1 to 1 solution.
3. Magnesium oxide.
4. Standard acid and alkali solutions.

Analytical Process.—Weigh out in duplicate ½ gram of the sample and place in a 500-cubic centimeter Kjeldahl flask. Add

30 cubic centimeters of distilled water and 10 grams of reduced iron. Allow to stand with frequent shakings for about 10 minutes, to insure solution of the soluble salts. Now add 10 cubic centimeters of the 1 to 1 sulfuric acid solution and allow to stand until the violence of the reaction has moderated. Place a long-stemmed funnel in the neck of the flask to prevent mechanical loss and heat the solution slowly until the boiling point is reached, boil for 5 minutes and cool. Wash off the funnel and the neck of the flask, add approximately 75 cubic centimeters of neutral water and a small piece of paraffine. Add approximately 7 grams of heavy magnesium oxide and 65 cubic centimeters of concentrated sodium hydroxide. Connect with the distilling apparatus and distill in the same manner as for the regular Kjeldahl. The nitrogen represents the nitrate and the ammonium salts in the sample.

If the sample is fairly pure the ammonia may be determined in the usual manner, (see Ammonia, Ammonium Salts, page 96) and the nitrates determined by the phenoldisulfonic acid method (see Nitrate Nitrogen, page 134).

NITRATE NITROGEN

The nitrate nitrogen content of a soil represents the amount that is immediately available for plant use. If the amount is excessive it may indicate an alkaline condition. Nitrate nitrogen is usually formed by microorganisms oxidizing ammonia or ammonium compounds, see "Biological Oxidation of Nitrogenous Compound—to Nitrites and Nitrates" Section 4, page 191.

Preservation of Sample.—Nitrate nitrogen is readily soluble and quickly acted upon by microorganisms. As its amount in soils is very small it may be changed in a few hours into another form. It frequently happens that it is necessary to make determinations upon samples that are at a distance from the laboratory. In such a case the samples should be dried at once in the field, to prevent all bacterial action and possible loss or change. If this cannot be done, the biological activity may be controlled by the addition of $\frac{1}{2}$ to 1 cubic centimeter of toluene per 100 grams of sample. The container must be tightly sealed to prevent a loss by vaporization. This treatment will keep the samples 36 to 48 hours without appreciable change. The toluene is removed by thoroughly air-drying the sample. Other antiseptics as chloroform, carbon-disulfide, etc., may be used.

The phenol compounds and formalin should not be used, the former interfers with the reactions in the determination and the later will readily polymerize to sugars in the presence of lime water, thus making a dark-colored solution that prevents accurate readings.

QUALITATIVE TESTS

Diphenylamine Test for Nitrates

The presence of 1 part of nitrate nitrogen in 35,000,000 parts of solution may be detected, provided no oxidizing agents as nitrites, ferric chloride, etc., are present.

Reagent:

Dissolve 0.7 gram of diphenylamine in a mixture of 60 cubic centimeters of chemically pure sulfuric acid (specific gravity 1.84) and 28.8 cubic centimeters of distilled water. Cool the mixture and add 11.3 cubic centimeters of concentrated hydrochloric acid (specific gravity 1.20). Allow to stand overnight, decant into a brown bottle, keep well stoppered, and away from the light.

Making the Nitrate Test.—Place approximately 1 cubic centimeter of the solution to be tested in a test tube, add 1 drop of the above reagent and mix thoroughly. Allow approximately 2 cubic centimeters of concentrated sulfuric acid to run down the sides of the tube in such a manner that it will run under, and form a layer under, the solution to be tested. Agitate the tube gently so as to cause a slight mixing of the two solutions at the plane of contact. Place in the water bath at a temperature of 40°C. for 15 to 20 minutes. A color at the junction of the two liquids indicates the presence of nitrates. If the nitrate solution is not too dilute, the test may be carried out on the test plate, using loopfuls of the different reagents. In case that larger amounts of nitrates are present the heating on the water bath may be dispensed with. If the nitrate concentration is quite marked, the concentrated acid may be mixed with the reagent and allowed to flow under the solution to be tested in the manner described above.

Testing for Nitrates in the Presence of Nitrites.—The test for nitrates may be conducted in the presence of nitrites by destroying the nitrites. Add a concentrated solution of urea to a small amount of the liquid to be tested in a test tube. Now add, in

the bottom of the tube, by means of a pipette, a dilute solution of sulfuric acid.

$$CO(NH_2)_2 + 2NHO_2 = CO_2 + 3H_2O + 2N_2.$$

Test the solution in the manner described above.

QUANTITATIVE TESTS

In the case of soils and manures an analysis of nitrate nitrogen is made for the purpose of determining the availability of the nitrogen content. The amount is usually very small but is accurately determined by the colorimetric method, provided no interfering substances are present, according to the following reactions:

$$C_6H_5OH + 2H_2SO_4 = C_6H_3(OH)(HSO_3)_2 + 2H_2O$$

$$H_2SO_4 + 2KNO_3 = 2HNO_3 + K_2SO_4$$

$$C_6H_3(OH)(HSO_3)_2 + HNO_3 = C_6H_2(OH)(SO_3)_2(NO_2) + H_2O$$

$$C_6H_2(OH)(SO_3)_2(NO_2) + 3NH_4OH = \left\{ \begin{matrix} C_6(OH_4N)(SO_2ONH_4)_2NO_2 \\ \text{yellow color} \end{matrix} \right\} + 3H_2O$$

Colorimetric Determination of Nitrate Nitrogen:

The nitrate nitrogen content of the soil is usually determined by the colorimetric method[32] unless chlorides are present. The method is possible in the presence of small amounts of chlorides but is questionable when large amounts are present. It has been suggested that the principal reactions contributing to the loss of nitrates in the presence of chlorides are as follows:

$$NaCl + H_2SO_4 = HCl + NaHSO_4$$

$$NaNO_3 + H_2SO_4 = HNO_3 + NaHSO_4$$

$$HNO_3 + 3HCl = 2H_2O + Cl_2 + NOCl$$

$$4HNO_3 + Heat = 4NO_2 + 2H_2O + O_2.$$

Reagents:

1. *Phenoldisulfonic Acid.*—Dissolve 25 grams of phenol in 150 cubic centimeters of concentrated sulfuric acid. The acid should be chemically pure. Add 75 cubic centimeters of fuming sulfuric acid (13 per cent SO_3) and heat by placing the flask in boiling water for 2 hours. Cool, place in a brown bottle, keep well stoppered, and store in a dark place

2. *Alkali.*—Concentrated solutions of either potassium or sodium hydroxide, or ammonium hydroxide (specific gravity

0.90). If a dilute solution of ammonium hydroxide is desired, dilute with 2 volumes of distilled water.

3. *Standard Nitrate Solution.*—Dissolve 0.7221 gram of pure, dry, potassium nitrate in water and dilute to exactly 1,000 cubic centimeters. Each cubic centimeter of this solution will contain the equivalent of 0.1 milligram of nitrogen.

Preparation of Solution:

1. *Nitrate Salts.*—Dissolve a weighed amount of the salt in water, dilute to a convenient volume, take an aliquot, evaporate to dryness and determine colorimetrically as described below.

2. *Soil Nitrates.*—Weigh out 100 grams of sieved (20 mesh) air-dry soil, or an equivalent amount of moist soil. Place in 1,000-cubic centimeter shaker bottles, add 2 grams of precipitated chalk and 500 cubic centimeters of distilled water. If moist soil has been used the amount of water in the sample must be deducted from the amount to be added. Shake thoroughly for 20 minutes. Filter through a coarse grade filter paper, allowing as much soil as possible to run on the filter. Return the first 100 cubic centimeters of filtrate, which may contain some particles of soil, to the filter and obtain a clear filtrate. Pipette off 100 cubic centimeter aliquots into 250-cubic centimeter Erlenmyer flasks for the determination of nitrates.

If the solution is colored, remove color in the 100 cubic centimeter aliquot by the addition of 2 to 5 cubic centimeters of aluminum cream (see Soil Solution, page 82), or by adding copper sulfate and magnesium carbonate (see Nitrate Nitrogen in solutions containing chlorides, page 137). Filter through a moistened filter and wash thoroughly. Combine the filtrate and washings. If a cheap grade of paper is used for the above filtration it should be washed with acid to remove any calcium, which may absorb some of the nitrates, before filtering out the aluminum cream. If only a small amount of color is present, it may usually be removed by substituting 2 grams of calcium oxide for the precipitated chalk above.

Analytical Process.—Evaporate the aliquot, from the filtrate, to dryness on the hot plate, being careful to remove the flasks as soon as dry. Better results are secured by evaporating the last 20 cubic centimeters on the steam bath. At the same time evaporate two or more 10- or 20-cubic centimeter portions of the standard to dryness.

To the dried salts in the flask add, from a burette, 2 cubic centimeters of phenoldisulfonic acid and immediately rotate the flask so that all portions of the salts are brought in contact with the acid. Allow to stand for 10 minutes. Add 10 to 15 cubic centimeters of distilled water and sufficient alkali to develop the color. If concentrated ammonium hydroxide is used 15 cubic centimeters will be sufficient. If sodium or potassium hydroxide is used a copious precipitate will result, which will partially dissolve when the solution is diluted. The balance of the precipitate may be allowed to settle and the clear, supernatant solution decanted for the colorimetric readings. The precipitate apparently does not affect the color of the solution. The use of ammonia gives the clearer solution but is objectionable, due to its irritating effects.

Build the solution, containing the developed color, up to exactly 100 cubic centimeters with distilled water. At the same time and in the same manner develop the color in the standards and dilute to 100 cubic centimeters. Place the solutions in the colorimeter and compare those of unknown strength with the standard. The readings should be made as soon as possible after developing the color as the solutions tend to decolorize slightly on standing.

Calculation of Results.—Calculate results as follows: $K : R :: S : X$, when,

K = reading of the unknown solution in millimeters or cubic centimeters.

R = reading of the standard solution in millimeters or cubic centimeters.

S = concentration in milligrams nitrogen in 100 cubic centimeters of the standard.

X = concentration in milligrams nitrogen in 100 cubic centimeters of the unknown.

Assume that 10 cubic centimeters of the standard was evaporated to dryness, the color developed and built up to 100 cubic centimeters. This solution contains 1 milligram of nitrogen. The unknown is also built up to 100 cubic centimeters in the same manner. Assume that the standard, when placed in the colorimeter, was set at 25 millimeters and that the reading of the unknown was 17.5 millimeters. Then,

$$17.5 : 25 :: 1.0 : x$$

x = 1.429-milligrams of nitrogen.

Calculate the amount of nitrate nitrogen in the original sample.

It must be remembered that all variations such as, difference in weight of sample, amount of aliquot, change of dilution, change of position of standard on the colorimeter, etc., must be noted and recorded and the results adjusted accordingly. If the above procedure is varied then the following formula covering all variations must be used:

$$X = \frac{SDK}{WAU}100M$$

when,

X = milligrams of nitrate per 100 grams of soil.
W = grams of soil taken.
S = cubic centimeters of water added to the soil.
A = aliquot taken for evaporation.
D = cubic centimeters to which A was diluted.
K = reading in millimeters of standard solution.
U = reading in millimeters of unknown solution.
M = milligrams of nitrate nitrogen in 1 cubic centimeter of the standard as diluted for reading.

If the comparisons are made in comparator tubes the volume, in cubic centimeters of solution may be substituted for millimeters above.

Determination of Nitrate Nitrogen in Solutions Containing Small Amounts of Chlorides:

Small amounts of chlorides in the soil solution may be removed by the addition of silver sulfate.[17]

Reagents:

1. $\frac{N}{1}$ *Copper Sulfate Solution:* 124.85 grams $CuSO_4 \cdot 5H_2O$ is dissolved in water and built up to 1,000 cubic centimeters.

2. *Silver Sulfate Solution:* 4 grams of silver sulfate is dissolved in water and built up to 1,000 cubic centimeters.

3. Calcium Hydroxide.

4. Magnesium Carbonate.

Analytical Process.—Weigh out 50 grams of sieved soil (25 grams in case of peat) place in shaker bottles, add 250 cubic centimeters of distilled water containing 5 cubic centimeters of $\frac{N}{1}$ copper sulfate, to remove any color, and sufficient silver sulfate to precipitate all the chlorides. Ten cubic centimeters of the above silver sulfate solution will remove 80 P.P.M. of chlorine in the above 250 cubic centimeters. If the soil is slighty acid and

does not give a colored extract, add 0.4 gram of calcium hydroxide and 1 gram of magnesium carbonate, to precipitate the copper and silver and also to aid in clarifying, and shake 10 or more minutes. Filter on a dry filter, discarding the first 20 cubic centimeters. Evaporate an aliquot to dryness and determine the nitrates colorimetrically as above.

If the soil is very acid or gives a colored extract, allow to settle after the first shaking of 10 minutes and decant 150 cubic centimeters of the supernatant liquid into a flask. Add to this flask, 0.2 gram of calcium hydroxide and 0.5 gram of magnesium carbonate, shake 5 minutes and filter as before, or add 1 gram magnesium oxide and heat to boiling with frequent shaking. Filter, cool, evaporate an aliquot, and determine colorimetrically as above.

In case of highly colored soil extracts, that cannot be decolorized by the copper sulfate, add "G Elf" carbon black, as directed under "Soil Solution," before adding the calcium oxide and magnesium carbonate. If the soil is calcareous, an additional amount (5 cubic centimeters) of copper sulfate should be added to insure enough copper hydroxide to remove completely the collodial carbon on filtration.

Titrametric Determination of Nitrate Nitrogen:

The titrametric method depends upon the reduction of the nitrate to ammonia by means of nascent hydrogen, produced by the action of an acid or alkali on a metal.

$$NaNO_3+4H_2 = NaOH+NH_3+2H_2O.$$

Modified Devarda Method

Reagents:

1. Approximately 5 per cent (by weight) hydrochloric acid solution.

2. *Devarda Alloy:* 50 per cent aluminum, 45 per cent copper and 5 per cent zinc. This mixture, of the proper degree of fineness, may be purchased from any chemical supply house.

3. Standard acid and standard alkali.

Analytical Process.—Place 100 grams of air-dry soil, or its equivalent of moist soil, in 500-cubic centimeter shaker bottles, add 300 cubic centimeters of the hydrochloric acid solution, shake 2 hours, and allow to stand overnight. Draw off 200 cubic centimeters of the supernatant solution and place in 800-cubic

centimeter Kjeldahl flasks containing 5 grams of sodium peroxide. Boil the contents of the flask down to approximately 25 cubic centimeters, or if urea is present, as in the case of manures, to exact dryness. The flask is not connected with the distilling apparatus during the boiling process. Add 200 cubic centimeters of distilled water, 0.5 gram of Devarda alloy (20 mesh), connect with the distilling apparatus and distill. One hundred twenty-five to one hundred fifty cubic centimeters should be distilled over, catching the distillate in $\frac{N}{20}$ acid. Complete the determination the same as for ammonia. If a distinct neutral point cannot be secured with the usual indicators use a few drops of rosalic acid as the indicator.

NITRITE NITROGEN

Nitrite nitrogen is seldom found in the soil. It may occur where there is a vigorous biological oxidation of ammonium compounds or a biological reduction of nitrates.

Test for Nitrites (Tromsdorf's test)

Reagents:

Make a paste of 4 grams of starch and dilute to approximately 100 cubic centimeters. Dissolve 20 grams of zinc chloride in 100 cubic centimeters of distilled water and bring to a boil. Add the boiling zinc chloride solution to the starch solution, slowly and with constant stirring. Continue heating the mixture until the starch is dissolved as much as possible and the solution nearly clear. Dilute to about 500 cubic centimeters and add 2 grams of zinc iodid, dilute to 1,000 cubic centimeters, filter and store in well-stoppered bottles in the dark.

Making the Test for Nitrites.—Place 3 drops of the Tromsdorf's reagent on a test plate, add 1 drop of dilute (1 to 3) sulfuric acid and 1 drop of the solution to be tested. A blue color indicates the presence of nitrites.

Determination of Nitrites:

Greiss Colorimetric Method

Reagents:

1. Dissolve 0.5 gram of sulphanilic acid in 150 cubic centimeters of dilute acetic acid (specific gravity 1.04).

2. Boil 0.1 gram of alpha-naphthylamine in 20 cubic centimeters of distilled water and filter through a plug of absorbent

cotton into 180 cubic centimeters of dilute acetic acid (specific gravity 1.04).

3. When ready to use mix equal parts of solution No. 1 and solution No. 2.

4. *Standard Sodium Nitrite Solution.*—Dissolve 0.0836 gram of pure silver nitrite in distilled water, add a solution of sodium chloride until all silver is precipitated. Make up to exactly 250 cubic centimeters, shake and allow to stand until the precipitate has settled. Dilute 10 cubic centimeters of the supernatant solution to 100 cubic centimeters. Each cubic centimeter of this dilution contains 0.01 milligram of nitrogen.

5. *Standard Colorimetric Solution.*—Dilute 10 cubic centimeters of the standard sodium nitrate solution to about 50 cubic centimeters, develop the color as described below and build up to 100 cubic centimeters.

Analytical Process.—To the solution to be tested add 16 cubic centimeters of the mixed 1 and 2 solutions as described in 3 above and dilute to 100 cubic centimeters. Allow the color to develop fully, about 5 minutes, and determine the depth of color produced by comparison with the standard in the colorimeter. Calculate the same as directed for nitrate nitrogen, colorimetric method.

PHOSPHORUS

Phosphorus occurs in rocks, soils, and plants in a variety of forms, none of which can be determined directly, due to the interference of carbon, iron, silicon, and other elements. Phosphorus combinations are easily decomposed at temperatures high enough to destroy organic matter, and consequently may be lost through volatilization, therefore some material is added to oxidize the phosphorus to a stable compound. There are a number of methods in use, the most satisfactory of which is the magnesium nitrate.

$$\begin{aligned} MgNO_3 + \text{Heat} &= MgO + NO_2 \\ 2NO_2 + C &= N_2O + CO_2 + O. \end{aligned}$$

The organic matter is destroyed in the above reactions, at the same time the following is taking place:

$$\left.\begin{aligned} 2P_2 + 5O_2 &= 2P_2O_5 \\ \text{or} \qquad & \\ P_2O_3 + O_2 &= P_2O_5 \end{aligned}\right\} P_2O_5 + 3H_2O = 2H_3PO_4$$

$$2MgO + 2H_3PO_4 = Mg_2P_2O_7 + 3H_2O.$$

The magnesium-pyro-phosphate is non-volatile and insoluble in water.

Determination of Total Phosphorus:

Reagents:

1. Concentrated nitric acid.
2. Dilute nitric acid, 1 to 3 dilution.
3. Concentrated hydrochloric acid.
4. *Magnesium Nitrate Solution:* 346 grams of magnesium nitrate ($Mg(NO_3)_2$) dissolved in 1,385 cubic centimeters of distilled water. Each cubic centimeter contains 0.2 gram of $Mg(NO_3)_2$.
5. Ammonium hydroxide.
6. *Ammonium Nitrate Solution:* Dissolve 400 grams of ammonium nitrate (NH_4NO_3) in distilled water and make up to 1,000 cubic centimeters. Five cubic centimeters of this solution are equivalent to two grams of the salt.
7. *Ammonium Molybdate Solution:* Dissolve 100 grams of molybdic acid in dilute ammonium hydroxide (144 cubic centimeters ammonium hydroxide (specific gravity 0.90) and 271 cubic centimeters distilled water); pour this solution slowly, and with constant stirring, into dilute nitric acid (480 cubic centimeters of nitric acid (specific gravity 1.42) and 1,148 cubic centimeters of distilled water). Keep in a warm place for several days or until a portion heated to 40°C. deposits no yellow crystals of ammonium molybdate. Decant the solution from any sediment and preserve in glass-stoppered bottles.

The solution may be prepared from chemically pure ammonium molybdate as follows: Dissolve 217.5 grams of ammonium molybdate in 830 cubic centimeters of distilled water and add twice the amount of nitric acid solution in the above. Allow to stand and decant as above.

Analytical Process.—If the sample is a mixed fertilizer, soil, or plant residue, weigh out 2 grams of the dry material, place in a 50-cubic centimeter evaporating dish, add the equivalent of 1½ to 2 grams of magnesium nitrate, evaporate to dryness on the steam bath, and ignite at a dull cherry red until the organic matter is destroyed.

If the sample is free of organic matter (rock phosphate or acid phosphate) the addition of magnesium nitrate and subsequent ignition may be omitted. In this case bring the weighed material into solution as described below.

Add to the ignited sample, or the unignited mineral, 15 cubic centimeters of concentrated nitric acid and 5 cubic centimeters of concentrated hydrochloric acid, cover with a watch-glass and digest on the steam bath or over a low flame for 30 minutes. If the flame is used it should be so adjusted that the solution will be as close as possible to the boiling point without actually boiling. This converts all the phosphorus compounds to orthophosphoric acid, soluble in water.

$$Mg_2P_2O_7 + 4HNO_3 = H_4P_2O_7 + 2Mg(NO_3)_2$$
$$H_4P_2O_7 + H_2O = 2H_3PO_4.$$

Cool, wash off the watch-glass with a jet of water, and transfer the contents of the evaporating dish to a 250-cubic centimeter volumetric flask, using distilled water. Fill flask approximately full and shake well, make up to mark, and again shake. Allow all dehydrated silica to settle out. Draw off with a pipette the following aliquots of the clear solution:

Original Material	Aliquot, Cubic Centimeters
Raw rock phosphate	25
Acid phosphate	50
Mixed fertilizers or grains	50 to 75
Soils, manures, or plant residues	100

Place each portion of the clear solution in a 250-cubic centimeter Erlenmyer flask and add a small piece of litmus as an indicator. From a burette, add just a sufficient amount of ammonium hydroxide to turn the paper blue. Now add one or two drops of dilute nitric acid (1 to 3 dilution) or just enough to turn the litmus a faint pink. The amount of acidity should be very carefully adjusted. Add approximately 2 grams of ammonium nitrate, in solution, or if desired the dry salt may be used.

Heat to approximately 65°C. on the water bath and add slowly, with constant stirring 25 to 50 cubic centimeters of ammonium molybdate solution. The amount of molybdate solution required will vary according to the character of material being analyzed. Avoid too great an excess as it merely wastes the reagent.

The phosphorus will be precipitated as the yellow, ammonium-phospho-molybdate.

$$21HNO_3 + H_3PO_4 + 12(NH_4)_2MoO_4 =$$
$$(NH_4)_3PO_4 \cdot 12MoO_3 + 21NH_4NO_3 + 12H_2O.$$

Test for complete precipitation by filtering a small amount of the solution and adding 1 to 2 cubic centimeters more of the molybdate solution.

Hold at 65°C. for 1 hour, with frequent shakings. From this point the determination may be made either gravimetrically or volumetrically, the later is best adapted for general laboratory procedure.

Volumetric Determination of Phosphorus:

Filter the precipitate by decantation on a close-textured filter, leaving as much of the precipitate as possible in the flask. Wash free of acid with cold ammonium nitrate solution (10 grams of the salt dissolved in 1,000 cubic centimeters or 25 cubic centimeters of the stock solution built up to 1,000 cubic centimeters). At least six to eight washings will be required before testing for the disappearance of acidity. The precipitate in the flask is washed at the same time by the addition of 10 to 15 cubic centimeters of the wash solution, the precipitate is then allowed to settle, and the solution decanted into the filter. Test the filtrate from the last washing with a few drops of methyl orange indicator, if it tests acid continue the washings until all trace of acid disappears. The final washing may be made with distilled water.

When the ammonium-phospho-molybdate precipitate is free of acid, transfer the filter paper, containing the precipitate, to the flask in which the precipitation was made. Add 10 to 20 cubic centimeters of distilled water and a few drops of phenolphthalein indicator. Break up the filter paper, either by agitation or with a stirring rod. Add sufficient $\frac{N}{10}$ sodium hydroxide solution to produce a deep, permanent red color, then add 2 to 5 cubic centimeters in excess. Record the exact amount added.

$$(NH_4)_3PO_4 \cdot 12MoO_3 + 23NaOH = 11Na_2MoO_4 + (NH_4)_2\,MoO_4 + NaNH_4HPO_4 + 11H_2O.$$

Stopper the flask and shake thoroughly. Titrate the excess of alkali with $\frac{N}{10}$ nitric or hydrochloric acid.

Calculations.—From the above reaction it is clear that each combining weight of phosphorus will require 23 molecules of sodium hydroxide, or 920 grams of sodium hydroxide, to neurtal-

ize 31 grams of phosphorus, but 1,000 cubic centimeters $\frac{N}{1}$ NaOH contain 40 grams. By proportion we find that:

$$920 : 31 :: 40 : x \quad x = 1.3478.$$

Therefore, 1,000 cubic centimeters $\frac{N}{1}$ NaOH are equivalent to 1.3478 grams of phosphorus, or 1 cubic centimeter of $\frac{N}{10}$ sodium hydroxide is equivalent to 0.13478 milligram of phosphorus. The number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide neutralized by the ammonium-phospho-molybdate precipitate multiplied by the factor 0.13478 milligram will give the milligrams of phosphorus in the aliquot.

Gravimetric Determination:

If the precipitate shows that it has been contaminated by some materials that have not been removed, the phosphorus may be determined gravimetrically.

Reagents:

1. *Dilute Ammonium Hydroxide:* 100 cubic centimeters of ammonium hydroxide (specific gravity 0.90) diluted to 1,000 cubic centimeters.

2. *Magnesia Mixture.*—Dissolve 22 grams of recently ignited calcined magnesia in dilute hydrochloric acid, avoiding an excess of the latter. Add a little calcined magnesia in excess and boil a few minutes to precipitate any iron, aluminum, and phosphorus; filter; add 280 grams of ammonium chloride, 261 cubic centimeters of ammonium hydroxide (specific gravity 0.90) and dilute to 2,000 cubic centimeters. Instead of the solution of 22 grams of calcined magnesia, 110 grams of crystallized magnesium chloride ($MgCl_2 \cdot 6H_2O$) dissolved in water, may be used. Add 280 grams of ammonium chloride and proceed as above.

Analytical Process. After the precipitated ammonium-phospho-molybdate has been washed free of acid as directed above, dissolve the precipitate on the filter by pouring equal parts of ammonium hydroxide and hot water on it. Receive in the original flask and dissolve the precipitate in the flask. Keep the volume to something less than 100 cubic centimeters. Nearly neutralize with hydrochloric acid, cool, add, drop by drop (one

drop per second) from a burette, 10 cubic centimeters of magnesia mixture, stirring vigorously all the time. After 15 minutes add 30 cubic centimeters ammonium hydroxide (specific gravity 0.90) and allow to stand until the supernatant liquid is clear (2 hours is usually long enough). Filter on an ashless filter, washing with dilute ammonium hydroxide, until the washings are practically free of chlorine, and dry the precipitated magnesium ammonium phosphate on the paper.

Transfer the main bulk of the precipitate to a glazed paper, ignite the filter paper in a tared crucible, transfer the precipitate to the crucible, bring gradually to the full heat, and ignite until white. If the precipitate appears gray, add a few drops of concentrated nitric acid, dry, and again ignite. Heat to constant weight. On heating, the magnesium ammonium phosphate is converted to magnesium pyrophosphate in which form it is weighed.

$$2MgNH_4PO_4 = 2NH_3 + H_2O + Mg_2P_2O_7.$$

Calculate the amount of phosphorus in the aliquot.

Determination of Phosphorus in Soils:

A quick method for determining phosphorus in soils, suggested by Dr. E. Truog, has been in use in the Soil Survey laboratories of Iowa State College for several years.

Reagents:

Same as for the determination of total phosphorus.

Analytical Process.—Weigh 2 grams of dry soil into 50-cubic centimeter evaporating dishes. Cover with 20 per cent magnesium nitrate solution and evaporate solwly on the steam bath or hot plate. Ignite at dull red in the electric muffle, cool and grind thoroughly in the dish, with a porcelain pestle. Add 10 cubic centimeters of concentrated nitric acid, heat slowly on the hot plate, adjusting the heat so that the boiling temperature will be reached in 10 minutes and simmer for exactly 5 minutes. A longer heating will result in an interference by silicon. Add an equal volume of water and filter at once. Wash two to three times with hot water, using such an amount that the filtrate will be 50 to 75 cubic centimeters, add 2 to 5 grams of ammonium nitrate and heat to 65°C. Add 15 to 25 cubic centimeters ammonium molybdate solution and keep at 65°C. for 1 hour and at

room temperature for 2 hours. Do not allow to stand overnight. Filter and determine volumetrically as described above.

FORMS OF PHOSPHORUS

The value of phosphorus in fertilizers or soils depends upon its solubility. The water soluble forms are believed to represent the primary ($M(H_2PO_4)$), the weak acid soluble the secondary or reverted ($M_2(HPO_4)$), and the weak acid insoluble the tertiary ($M_3(PO_4)$).

Determination of Water-soluble Phosphorus:

In Fertilizers.—Place 2 grams of the sample on a 9-centimeter filter, wash with successive small portions of water, allowing each portion to pass through before adding more, until the filtrate measures about 250 cubic centimeters. If the filtrate is turbid, add a little nitric acid.* Proceed as directed under Determination of Total Phosphorus, page 142, beginning with—"Draw off with a pipette the following aliquots of the clear solution."

In Soils.—Place 100 grams of air-dry soil in a shaker bottle and add 500 cubic centimeters of distilled water, shake 2 hours and allow to stand overnight in an inclined position. Shake ½ hour and filter. Evaporate 100 to 200 cubic centimeter aliquots to dryness and proceed as directed under the determination of $\frac{N}{5}$ nitric acid-soluble phosphorus below.

As the water-soluble phosphorus in soils may be held by absorption, larger amounts may be secured by using the "replacement" method. The same solution and the same procedure are used as directed for the determination of adsorbed potassium. In this case the solution is treated with magnesium nitrate, ignited and determined as directed under total phosphorus.

Determination of Weak Acid-soluble Phosphorus:

In Fertilizers.—Drop the filter, from the water-soluble determination, into 100 cubic centimeters of 2 per cent citric acid solution, previously heated to 65°C., shake thoroughly and keep at this temperature for 30 minutes. The flask should be loosely stoppered to prevent evaporation. Filter, wash, make up to a convenient volume, draw off an aliquot, add magnesium nitrate solution, evaporate to dryness, and proceed as directed for total phosphorus.

* If any organic matter is present it must be destroyed by evaporating to dryness and igniting the same as for total phosphorus.

The 2 per cent citric acid is frequently replaced with neutral ammonium citrate (see below).

In Soils.—The determination of weak acid-soluble phosphorus in soils is made by the use of 2 per cent citric acid, neutral ammonium citrate, or with $\frac{N}{5}$ nitric acid. The 2 per cent citric acid determination follows the same procedure as the neutral ammonium citrate.

NEUTRAL AMMONIUM CITRATE SOLUBLE PHOSPHORUS IN SOILS

Reagents:

1. Same as for total phosphorus.

2. *Neutral Ammonium Citrate.*—Dissolve 370 grams of commercial citric acid in 1,500 cubic centimeters of distilled water, nearly neutralize with commercial ammonium hydroxide, cool, add ammonium hydroxide until exactly neutral, testing with litmus, azolitmin, rosalic acid indicator, or with the hydrogen-ion test, and dilute to make the specific gravity 1.09 at 20°C. (Association Official Agricultural Chemists 6, p. 390, 1922–1923).

Analytical Process.—Weigh out 25-gram portions of air-dry soil in 250-cubic centimeter volumetric flasks and add, with frequent shakings, sufficient neutral ammonium citrate solution to make up to the mark, stopper, and place in the water bath, heat to 65°C. for 2 hours shaking frequently. Allow to settle overnight. Draw off 100-cubic centimeter aliquots, if not clear filter and wash, place in evaporating dishes, add magnesium nitrate, evaporate to dryness and proceed as directed under total phosphorus. If the dishes are placed under the hot plate during the process of evaporation little trouble will be experienced with the material boiling over.

$\frac{N}{5}$ NITRIC ACID SOLUBLE PHOSPHORUS IN SOILS

Determine the neutralizing power of 10 grams of the soil to be tested by adding 100 cubic centimeters of $\frac{N}{5}$ nitric acid and digesting for 5 hours at 40°C. and titrating an aliquot of the clear filtrate. Calculate the strength of acid solution required to make 1,000 cubic centimeters after allowing for the quantity of soil to be neutralized.

Analytical Process.—Place 25 grams of air-dry soil in 250-cubic centimeter volumetric flasks, add the corrected acid solu-

tion, corrected so that it will be $\frac{N}{5}$ after digestion, and fill to the mark. Digest at 40°C., shaking every ½ hour for 5 hours. Filter the entire content of flasks, pouring the filtrate back until it becomes clear. Evaporate an aliquot to dryness in a porcelain dish, and 1 to 2 cubic centimeters of nitric acid to oxidize any organic matter, moisten the residue with hydrochloric acid, digest with water and filter into a 250-cubic centimeter flask. Add 2 to 5 grams of ammonium nitrate, then strong ammonium hydroxide until a permanent precipitate forms. Add concentrated nitric acid, drop by drop, until the precipitate just dissolves. Precipitate the phosphorus with ammonium molybdate solution at 80°C. keep on the water bath 15 minutes, then allow to stand 10 minutes before filtering. Filter and wash free of acid with ammonium nitrate solution and determine the phosphorus gravimetrically using magnesia mixture.

Determination of Organic Phosphorus in Soils:

As a part of the phosphorus in the soil is in the organic form, special methods are necessary to determine the fraction.[29]

Analytical Process.—Make an alkaline extraction of one part of soil with four parts of 2 per cent ammonium hydroxide, shaking 1 hour then centrifuging to obtain a clear extract. If other solvents are used for extraction the solution is made ammoniacal after centrifuging. Add sufficient ammonium chloride to the clear solution to make a 5 per cent solution. Precipitate the phosphorus by the addition of magnesia mixture in excess. Allow to stand 3 hours. Draw off the clear solution, filter the precipitate, and wash with water containing a trace of the magnesia mixture. Collect the precipitate in about 150 cubic centimeters of distilled water and add 8 cubic centimeters of concentrated nitric acid for each 100 cubic centimeters of the original solution. Neutralize with ammonia, concentrate, under reduced pressure, to about 125 cubic centimeters. Add 5 to 8 cubic centimeters of concentrated nitric acid and 100 cubic centimeters of ammonium molybdate solution. Place in the water bath and keep at 60°C. for 15 minutes, then at room temperature for 2 hours. Filter, wash free of acid with ammonium nitrate solution, dissolve in 2 per cent ammonia, add 20 grams ammonium chloride and 50 cubic centimeters of magnesia

mixture. Let stand 3 hours, filter, wash, dissolve in nitric acid and evaporate to dryness to dehydrate the silica. From this point the phosphorus is determined volumetrically as directed under total phosphorus.

PREPARATION OF ACID PHOSPHATE

Place two 5-gram portions of the stock sample of raw rock phosphate in 50-cubic centimeter evaporating dishes and add slowly, stirring constantly, sufficient sulfuric acid to convert all the insoluble phosphorus to the soluble form according to the following reaction. If the total phosphorus content of the sample is known, calculate the amount of acid required as directed below, diluting this amount with about one-fourth its volume of water.

$$Ca_3(PO_4)_2 + 2H_2SO_4 = Ca(H_2PO_4)_2 + 2CaSO_4.$$

The reaction takes place in two stages as follows:

$$Ca_3(PO_4)_2 + 3H_2SO_4 = 2H_3PO_4 + 3CaSO_4.$$

$$Ca_3(PO_4)_2 + 2H_3PO_4 = Ca(H_2PO_4)_2 + Ca_2H_2(PO_4)_2$$

$$Ca_2H_2(PO_4)_2 + H_2SO_4 = Ca(H_2PO_4)_2 + CaSO_4.$$

The mixing should be done under the hood to avoid the irritating hydrofluoric acid fumes that may arise. The raw rock phosphate usually consists of a mixture of calcium phosphate with traces of fluorine and chlorine.

$$2Ca_3(PO_4)_2 \cdot Ca_2F(PO_4)_2 + 7H_2SO_4 = 3CaH_4(PO_4)_2 + 7CaSO_4 + 2HF.$$

If much HF is formed, transfer the mixture to a small board and set aside for a few days. Test qualitatively for the various forms of phosphorus in both the original and treated samples. Make an analysis of the various forms in both the treated and original samples and compare results.

Calculations.—Assume that the original sample of raw rock phosphate contained 9 per cent total phosphorus, then 5 grams would contain 0.45 gram of the element P. Assuming that all the phosphorus in the sample is in the form of tri-calcium phosphate the total amount would be in the following proportion:

$$P : Ca_3(PO_4)_2 :: 0.45 : x$$

substituting the molecular weights, then

$$31 : 310.3 :: 0.45 : x$$

$x = 4.504$ grams of $Ca_3(PO_4)_2$ in the 5-gram sample.

In the above reaction, 1 molecule of tri-calcium phosphate combines with 2 molecules of sulfuric acid, consequently the amount of sulfuric acid required may be expressed proportionally:

$$310.3 : 196 :: 4.504 : x$$

$$x = 2.84 \text{ grams of sulfuric acid.}$$

POTASSIUM

Many methods have been suggested for the determination of potassium, the most satisfactory, however, is the J. Laurence Smith method. This method allows the determination of both sodium and potassium in the same sample.

Determination of Sodium and Potassium in Soils:

Reagents:

1. Pure ammonium chloride.
2. Pure calcium carbonate.
3. Hydrochloric acid.
4. Ammonium hydroxide.

5. *Ammonium Oxalate Solution.*—Dissolve 142 grams of ammonium oxalate in 1,700 cubic centimeters of distilled water. When solution is complete add 50 cubic centimeters of ammonium hydroxide, or, dissolve 126 grams of oxalic acid in 1,700 cubic centimeters of distilled water and add 270 cubic centimeters of 28 per cent ammonium hydroxide.

6. Ten per cent barium chloride solution.

7. *Platinic Chloride Solution.*—A solution containing the equivalent of 1 gram of metallic platinum (2.1 grams of H_2PtCl_6) in every 10 cubic centimeters.

8. *Ammonium Chloride Solution.*—Dissolve 200 grams of ammonium chloride in 1,000 cubic centimeters of distilled water, add 5 to 10 grams of pulverized, potassium platinic chloride and shake at intervals for 6 to 8 hours. Allow to stand overnight, filter or decant, and store in stoppered bottles. The residue may be used for the preparation of a fresh supply.

9. *Alcohol Wash* (80 per cent alcohol) made by diluting 95 per cent alcohol with water to a specific gravity of 0.8645 at 15.6°C. or by the use of the alcoholometer. The alcohol may be denatured before diluting as follows:[37]

. . to every 100 parts of ethyl alcohol (not less than 180 degrees proof) add 10 parts of wood alcohol (methyl) (specific gravity 0.830 at 60 F.) and one-half part of benzine. If the benzine is derived from

petroleum it must have a specific gravity of not less than 0.8000. If derived from coal tar it must have a boiling point of not less than 150 or more than 200°F.

In place of the above the following may be used:

. . . to every 100 parts of ethyl alcohol (not less than 180 degrees proof) add 10 parts of methyl alcohol (specific gravity 0.830 at 60°F.) and one-half part of pyridine base.

10. *Acidulated Alcohol.*—Add 230 cubic centimeters of hydrochloric acid (specific gravity 1.20) to 3,000 cubic centimeters of 95 per cent alcohol. Place in a tall bottle, fitted with a tube extending to the bottom. Arrange a chlorine generator by fitting a 2,000-cubic centimeter flask with a two-holed rubber stopper, equipped with a dropping funnel and a short exit tube. Fill the flask one-fourth full with sodium chloride, connect the exit tube with the tube in the alcohol, and generate chlorine by dropping concentrated sulfuric acid on the sodium chloride. The gas is conducted into the mixture until it shows a normality of 2.25 by titration.

Analytical Process.—Weigh out accurately ½ to 1 gram of soil and place in an agate mortar. Add 1 gram of ammonium chloride and 2 grams of calcium carbonate. Grind until thoroughly mixed and all grittiness is gone. Prepare a platinum crucible (8 centimeters long by 2.5 centimeters in diameter; any deep platinum or nickle crucible will do) by placing in the bottom 2 grams of calcium carbonate and gently tapping to insure an even distribution over the bottom. Next add the mixture containing the soil and cover with a layer of 2 to 4 grams of calcium carbonate. The crucible should be one-half to three-fourth full. Make a support for the crucible by cutting a hole in a stiff piece of asbestos, so that the crucible will rest in a tilted position. Fix the crucible in the support so that the upper one-fourth will not come in direct contact with the flame. Cover the crucible and heat slowly, taking care to keep the upper part and cover as cool as possible. Adjust the heat so that no white fumes are given off. After approximately 15 minutes, increase the heat slowly, when the odor of ammonia ceases to come off, use the blast and heat for 40 to 50 minutes.

$$2KAlSi_3O_8 + 5CaCO_3 + CaCl_2 = 2KCl + Al_2O_3 + 6CaSiO_3 + 5CO_2$$

Allow the crucible containing the fusion to stand until cold, then empty into a casserole. All of the material will usually come out readily. If necessary, place the crucible in the casserole and heat on the steam bath to slack out any adhering particles. Wash the lid of the crucible allowing the washings to flow into the casserole. Build the contents of the casserole up to approximately 250 cubic centimeters and heat for 20 to 30 minutes. When all has dissolved that will go into solution, decant the supernatant liquid into a filter and grind the residue thoroughly, in an agate mortar, until all grittiness is gone. Wash this residue back into the casserole with hot water and heat until all is dissolved that will be, by this treatment. Wash all the material into the filter and wash the filtrate until free of chlorides. Combine the washings and the filtrate and evaporate to about 200 cubic centimeters.

In testing for chlorides, remember that in an alkaline solution, silver nitrate is reduced to silver oxide, giving a brownish precipitate. Test a couple of drops of the filtrate in a test tube with 5 per cent silver nitrate; if a brownish precipitate appears, add a few drops of nitric acid until the brown color disappears. If the solution is milky more washing is required.

Removal of Calcium.—Make the concentrated alkaline filtrate ammoniacal by the addition of 30 to 40 cubic centimeters of concentrated ammonium hydroxide, add approximately 35 cubic centimeters of ammonium oxalate solution to precipitate the calcium. Filter, wash free of chlorides, evaporate the filtrate to about 100 cubic centimeters, add a drop of barium chloride solution to precipitate any possible sulfur, precipitate the excess barium by the addition of more ammonium oxalate, filter and wash free of oxalates. Evaporate to dryness and heat carefully until all ammonium salts are driven off. Cool, take up with hot water and a little hydrochloric acid, and again evaporate to remove all trace of ammonium salts. Take up the residue with hot water and a little hydrochloric acid. Filter into a weighed platinum crucible, evaporate to dryness at a temperature of 105 to 110°C., and weigh as a mixture of sodium chloride (NaCl) and potassium chloride (KCl).

SEPARATION OF SODIUM AND POTASSIUM

Take up the salts with a little hot water to which has been added 5 to 8 drops of hydrochloric acid. Wash into a 50-cubic

centimeter beaker or evaporating dish, add 0.5 to 2.5 cubic centimeters of platinic chloride solution and evaporate to a syrupy consistency on the steam bath. The precipitate consists of sodium platinic chloride (Na_2PtC_6) and potassium platinic chloride (K_2PtCl_6). The former is soluble in 80 per cent alcohol.

Prepare a Gooch filter as follows: Digest a quantity of long fiber asbestos with hot, 5 per cent, sulfuric acid and an excess of potassium permanganate. Wash, treat with oxalic acid in excess, and wash free of all soluble matter. Keep in an aqueous solution, tightly stoppered. Make a mat of the treated asbestos by pouring a small quantity of the suspension into the crucible, using gentle suction. Secure a firm mat over the bottom. Wash thoroughly with hot distilled water. Reduce the crucible to constant weight by heating at 105 to 110°C. for 2- to 3-hour periods, washing with 100 cubic centimeters of hot, distilled water after each weighing.

Drench the precipitate with 80 per cent alcohol and transfer to the Gooch using gentle suction. Wash the precipitate eight times, using 5 to 8 cubic centimeters of alcohol each time, allowing each portion to pass through before adding another. Do not allow the precipitate to become dry while washing. Dry the washed precipitate at 105 to 110°C. to constant weight. The precipitate is potassium platinic chloride (K_2PtCl_6). The amount of potassium chloride may be calculated by applying the factor 0.307. The difference between this weight and the weight of the mixed chlorides gives the weight of sodium chloride.

Determination of Potassium:

The sample is prepared for the determination in the same manner as directed for the Determination of Sodium and Potassium, proceeding to the point, Removal of Calcium.[2]

Analytical Process.—The combined washings and filtrate are evaporated to about 250 cubic centimeters and treated with a slight excess of hydrochloric acid (about 10 cubic centimeters (specific gravity 1.20)), the filtrate evaporated to dryness on the steam bath, taken up with about 50 cubic centimeters of hot, distilled water and filtered into a 150-cubic centimeter beaker. The precipitate is washed until the filtrate nearly fills the beaker. Add to the filtrate 0.5 to 2.5 cubic centimeters of platinic chloride solution and 1 to 1½ cubic centimeters of concentrated hydrochloric acid; place on the steam bath and evaporate to a syrupy

consistency as directed above. Add to the residue 15 to 20 cubic centimeters of acidulated alcohol, stirring until all the calcium chloride dissolves. The liquid is decanted into a previously weighed Gooch crucible, prepared as directed above, using gentle suction, and the precipitate is transferred to the crucible and washed three or four times with 80 per cent alcohol. The precipitate is now washed ten or twelve times with ammonium chloride solution and finally with ten or twelve washings of 80 per cent alcohol, care being taken to wash out all ammonium chloride that may stick to the sides of the crucible. Dry to constant weight and weigh as potassium platinic chloride as directed above.

A correction must be made for the amount of sodium and potassium in the reagents. Run a blank. The air in the room and the water used must be free of ammonia when the platinic chloride solution is used, as any ammonia taken up by the solution, from the air, will be weighed as ammonium platinic chloride.

DETERMINATION OF POTASSIUM IN FERTILIZERS:

Potassium may be in fertilizers in an organic form in such materials as cottonseed meal, tobacco stems, plant residues, etc., or it may be in the form of soluble salts of chlorides, sulfates, or combinations of these mixed with the chlorides of magnesium and sodium, or it may be in the form of ashes. The potassium must be brought into solution.

Preparation of Solution

Organic Compounds.—Saturate 10 grams of the sample with strong sulfuric acid and ignite at a low, red heat to destroy the organic matter. Add 2 to 3 cubic centimeters of hydrochloric acid, warm slightly to loosen the mass from the dish, add 20 to 25 cubic centimeters of distilled water, filter into a 250-cubic centimeter graduate flask, wash free of chlorides and make up to the mark. If the amount of potassium is small proceed as directed for Determination of Potassium. If the amount is large proceed as directed below.

Mixed Fertilizers.—Place 2.5 grams of the sample on a 12.5 centimeter filter and wash with successive portions of hot water until the filtrate amounts to about 200 cubic centimeters. Add 2 cubic centimeters of hydrochloric acid, heat to boiling, transfer to a 250-cubic centimeter volumetric and make up to the mark.

Potash Salts.—Dissolve 2.5 grams of the sample in water and dilute to 250 cubic centimeters in a volumetric flask.

Analytical Process.—Dilute 25 to 50 cubic centimeters of either of the above solutions, to approximately 150 cubic centimeters, heat to boiling and add, drop by drop, with constant stirring, a slight excess of 5 per cent barium chloride solution. Without filtering, add in the same manner, barium hydroxide solution in slight excess; filter while hot and wash until the precipitate is free of chlorine. Add to the filtrate 1 cubic centimeter of strong ammonium hydroxide and then a saturated solution of ammonium carbonate until the excess barium has been precipitated. Heat approximately to boiling and add, in fine powder, 0.5 gram of pure oxalic acid or 0.75 gram of ammonium oxalate; filter; wash free of chlorine; evaporate the filtrate to dryness in a platinum dish, and ignite carefully over the free flame, below a red heat, until all volatile matter is driven off. Digest the residue with hot water, filter through a small filter and dilute the filtrate if necessary, so that for each decigram of potassium oxide there will be at least 20 cubic centimeters of liquid. Acidify with ½ to 1 cubic centimeter of hydrochloric acid and add platinic chloride solution in excess. Proceed as directed under the Determination of Sodium and Potassium. If there is an appearance of foreign matter in the precipitate, (liable to occur in the case of soluble salts) the precipitate of potassium platinic chloride should be washed with the ammonium chloride solution as directed under the Determination of Potassium.

WATER-SOLUBLE POTASSIUM

Water-soluble potassium may be determined in manures, soils, etc. Fifty to one hundred grams of air-dry material, or its equivalent of moist material, is weighed out, placed in shaker bottles, and 500 cubic centimeters of distilled water added. Shake the mixture for 8 hours, allow to stand overnight, decant the supernatant liquid and take an aliquot of 100 to 200 cubic centimeters for the determination. If the solution is not clear, filter through a dry filter paper and wash. Place the aliquot in an evaporating dish, add 2 cubic centimeters of concentrated sulfuric acid and evaporate to dryness. Complete the determination as directed under Determination of Potassium.

ADSORBED POTASSIUM

Very little water-soluble potassium may be extracted from soils, due to the fact that it is apparently adsorbed. The adsorbed salts may be more or less successfully removed by the so-called "replacement methods."

Analytical Process.—Place 100 grams of air-dry soil in a 1,000-cubic centimeter shaker bottle and add 500 cubic centimeters of $\frac{N}{1}$ ammonium chloride solution, shake 3 hours, filter through a Pasteur-Chamberlain filter and take 100- to 200-cubic centimeter aliquot for analysis. Evaporate the aliquot to dryness, ignite carefully over the free flame, below a red heat, until all volatile matter is driven off, take up with hot water and filter into a 150-cubic centimeter beaker. Proceed as directed in the Determination of Potassium, beginning with: "The precipitate is washed until the filtrate nearly fills the beaker," page 153.

In some cases the adsorbed potassium is not all released on the first extraction. It may be necessary to extract the soil several times using fresh solutions each time, in which case the filtrates are combined and evaporated to a definite volume before the aliquot for analysis is taken.

Some workers prefer to use calcium chloride in place of ammonium chloride for the extraction.

Recovery of Platinum and Alcohol.—Wash the crucibles containing the potassium platinic chloride precipitate with about 150 cubic centimeters of hot, distilled water to dissolve all the salt and make the crucibles ready for the next determination. Keep the washings in a bottle marked "Platinum Washings, Water-soluble." Save all the alcohol washings, as they contain all the platinic chloride not converted into sodium, or potassium salts also contain the soluble sodium platinic chloride. Keep these washings in a glass-stoppered bottle marked "Platinum Washings, Alcohol Soluble."

Recovery of Alcohol.—Usually the recovery of alcohol takes so much time that it is not a profitable practice. In this case the alcohol washings are combined with the platinum washings, diluted with approximately an equal amount of distilled water, and the platinum precipitated as described under Recovery of Platinum, below. If desired to recover the alcohol proceed as follows: Place the washings in a 2,000- to 3,000-cubic centimeter flask, place on the electric hot plate, and distill to one-fourth

the original volume. If necessary to use gas, the distilling flask should be protected by placing it in a water bath and the receiving flask should be guarded to prevent vaporization and danger of ignition of the alcohol. Better results are secured by the use of a steam-heated copper still. Dilute the residue to three to four times its volume and recover the platinum as described under Recovery of Platinum, below.

The recovered alcohol is usually contaminated with ammonia, especially if the ammonium chloride wash has been used. In this case add just enough sulfuric acid to neutralize the ammonia and redistill.

The recovered alcohol will have a percentage of approximately 65. This may be increased by adding lumps of calcium oxide and redistilling.

Recovery of Platinum.—Place the solution containing the platinum residues in a tall cylinder or precipitating jar. As platinum is reduced best in dilute solutions, the color should never be stronger than a lemon yellow. Make the solution strongly acid by the addition of 20 to 25 cubic centimeters of hydrochloric acid per 1,000 cubic centimeters of solution. Add chemically pure mossy zinc, the equivalent of ½ to 1 gram per 1,000 cubic centimeters. The action should be violent, that is the solution should boil due to the evolution of hydrogen. The amount of zinc should be such that it will be just used up by the acid. The platinum salts will be decomposed by the nascent hydrogen into the basic chlorides and free platinum, the latter being deposited as platinum black. The precipitate will settle quickly. When clear, decant, wash the residue four to six times by decantation, then several times on a Buchner filter, using distilled water. Dry, weigh carefully, take up with aqua regia and bring into solution as platinic chloride.

Aqua regia reacts violently with platinum black, so add approximately 25 cubic centimeters of concentrated hydrochloric acid for each gram of platinum black. Heat until the fumes begin to rise, then add, drop by drop, one-third as much nitric acid, stirring constantly with a glass rod until all danger of boiling over ceases. Evaporate to about one-half the volume, build up again with aqua regia and boil to drive off the excess nitric acid. Allow to settle, decant the supernatant solution, wash the residue with hot water, to remove the crystals of platinic chloride formed around the metallic platinum, add the

washings to the decanted solution, treat the residue with more aqua regia, decant, add to the original decanted solution, dry the residue and weigh. Calculate the amount of platinum in solution and make the solution to the desired dilution.

All filter paper should be saved, ignited, and the platinum recovered with aqua regia as above.

SULFUR

The determination of sulfur in either plants or soil is dependent upon the oxidation of the combined sulfur to the sulfate and precipitating as an insoluble sulfate by the addition of barium salts, distinct from all other soil precipitates. The precipitated barium sulfate may be determined: (1) by filtering and weighing the precipitate; (2) by comparing the turbidity of the freshly prepared precipitated solution, in the colorimeter, with that of a standard similarly prepared; or (3) by determining the turbidity directly (photometrically) and calculating from a prepared scale.

Determination of Sulfur in Soil:

The method of Shaw and MacIntire[33] removes any contaminating barium at the start by precipitating it as the carbonate.

Reagents:

1. Chemically pure anhydrous sodium carbonate (Na_2CO_3)
2. Sulfur-free sodium peroxide (Na_2O_2).
3. Ethyl alcohol.
4. Concentrated hydrochloric acid.
5. Five per cent barium chloride solution.

Analytical Process.—Place 10 grams of finely ground soil (100-mesh) in a nickle crucible with an equal amount of sodium carbonate, mix thoroughly with a nickle stirring rod. Add carefully 4 cubic centimeters of distilled water and stir to a stiff paste. Add immediately 1-gram portions of sodium peroxide, stirring well after each addition to avoid frothing, until the mixture is dry and granular, then add as a surface coating enough sodium peroxide to make a total of 25 grams.

Fuse the mixture at 400 to 500°C. for ½ hour by placing on an electric hot plate, or in an electric oven, then raise the heat rapidly to 900°C. and heat for 10 minutes. Withdraw from the furnace quickly and manipulate so that the melt will spread out

in a thin layer over the interior of the crucible. Cool as rapidly as possible.

Immerse the cooled crucible sideways in distilled water in a 600-cubic centimeter beaker and add about 5 cubic centimeters of alcohol to decompose the sodium manganate. Cover the beaker with a watch-glass, place on a cold electric hot plate and apply heat, boil briskly until all the glassy green lumps are disintegrated (about 30 minutes), remove crucible and rod and wash off any adhering particles. Filter the flesh-colored flocculent suspension immediately through a 9-centimeter Buchner funnel using suction. Return the residue, and filter paper to original beaker, add about 1 gram of sodium carbonate, macerate with a stirring rod, add 50 to 75 cubic centimeters of distilled water and bring to a brisk boil, stirring vigorously. Again filter through a Buchner, suck nearly dry, and wash three or four times with 20-cubic centimeter portions of hot water. The volume of the combined filtrates should be about 700 cubic centimeters.

Cover the beaker, containing the filtrate, with a watch-glass and arrange a funnel with its stem bent so that it just reaches into the lip of the beaker. Add gradually 80 cubic centimeters of concentrated hydrochloric acid, taking care that it runs down the sides of the beaker. Slightly lift the cover and stir gently, watching for strong effervescence. If the solution does not effervesce, add more acid immediately until it does. Concentrate the volume to approximately 400 cubic centimeters.

If the concentrated solution is cloudy, dehydrate the silica by evaporating in a shallow 1,000-cubic centimeter porcelain dish. Permit evaporation and dehydration without stirring except occasionally to break the surface crust. Take up the dehydrated mass with ½ cubic centimeter hydrochloric acid and 200 cubic centimeters of distilled water, heating gently; filter and wash six times to a volume of 400 cubic centimeters.

From this point the sulfur may be determined gravimetrically or by the turbidity methods.

Gravimetric Determination.—Heat the concentrated clear solution to boiling, and add slowly enough 5 per cent barium chloride solution to precipitate all the sulfur. Allow to stand overnight in a warm place in order that the barium sulfate crystals will be as large as possible. Filter the warm solution through an ashless paper or filter through an asbestos pad in a tared Gooch. For preparation of asbestos and Gooch, see

"Potassium," page 153. If filtered through paper, wash several times with hot water, dry on the paper, place in a tared crucible, ignite to constant weight and weigh as barium sulfate ($BaSO_4$). If filtered through a Gooch, wash several times with hot water, using gentle suction, ignite to constant weight in the electric muffle, or place the Gooch in a porcelain crucible over a burner, ignite, cool and weigh as barium sulfate ($BaSO_4$).

TURBIDITY DETERMINATION

Turbidity determinations may be made either in the colorimeter or in a photometer. In the case of the colorimeter,[32] the turbidity of the unknown solution is compared with that of a solution, similarly prepared, containing a known amount of sulfate. In the case of the photometer, the depth of the turbid liquid required to extinguish a standard flame is taken as a measure of the amount of sulfur present. The photometer, with directions for its use and interpretation of results, may be purchased from any chemical supply house.

Determination of Turbidity of Sulfate Solutions:

(Using colorimetric procedure)

Reagents:

1. *Barium Chloride.*—Powdered or in small crystals.
2. *Hydrochloric Acid.*—Concentrated.
3. *Standard Potassium Sulfate Solution.*—Dissolve 0.9071 grams of pure potassium sulfate in water and dilute to 1,000 cubic centimeters. Each cubic centimeter of this solution contains 0.5 milligram of SO_4.
4. *Standard Turbidity Solution.*—Dilute 20 cubic centimeters of the standard sulfate solution to 1,000 cubic centimeters. Of this dilute solution use 100 cubic centimeters and put it into series with the test solutions. The turbidity of the standard solution is to be developed, as described under Analytical Process, at the same time and in the same manner as that of the unknown solutions.

Analytical Process.—Dilute or reduce the solutions containing the oxidized sulfur to a definite volume, 250 to 500 cubic centimeters, draw off 50- to 100-cubic centimeter aliquots and place in 250-cubic centimeter Erlenmyer flasks. Add one or two drops of hydrochloric acid and 0.1 to 0.2 gram of barium chlo-

ride crystals. Stopper with cork stoppers and shake vigorously at intervals during 30 to 40 minutes.

Compare the turbidity of the known and the unknown solutions in the colorimeter using the same procedure as directed in the Colorimetric Determination of Nitrates, page 136.

Determination of Sulfur in Organic Matter:

Reagents:

1. Same as for sulfur in soils.
2. Chemically pure sodium hydroxide.

Analytical Process.—Weigh out accurately 1- to 2-gram portions of the finely-ground, dry, organic matter and place on squares of glazed paper or in watch-glasses. Place in nickel crucibles 1- to 2-gram portions of chemically pure sodium hydroxide, using equivalent amounts of sample and alkali, and heat carefully on the electric plate until the hydroxide just melts. Place a 4-inch nickel stirring rod in each crucible, add the organic matter slowly, stirring constantly. Immediately add, very carefully, in amounts not to exceed ½ gram, small portions of sodium peroxide, until an amount equal to five times the weight of the sample has been added stirring thoroughly after each addition. Keep at the same temperature for ½ hour, then place in the electric muffle and fuse at approximately 900°C. for 20 to 30 minutes. Proceed as directed in Determination of Sulfur in Soil, page 159, beginning with: "Immerse the crucible sideways in distilled water in a 600-cubic centimeter beaker."

Determination of Water-soluble Sulfur:

Analytical Process.—Add the equivalent of 500 cubic centimeters of distilled water to 100 grams of air-dry soil. Shake 5 hours, filter through a Pasteur-Chamberlain filter. Draw off an aliquot, acidify with two to five drops of hydrochloric acid and precipitate the sulfur with barium chloride as described above.

Determination of Sulfur in Rain Water:

Sulfur may be added to soils by the action of rain water. It is added chiefly in the form of hydrogen sulfide, sulfur dioxide, or sulfur trioxide. These forms must be oxidized to the sulfate.[3]

Reagents:

1. *Hydrochloric Acid.*—Concentrated.
2. Five per cent barium chloride solution.

3. *Benedict's Solution.*—Dissolve 200 grams of copper nitrate and 50 grams of sodium or potassium chlorate in distilled water and build up to 1,000 cubic centimeters.

Analytical Process.—Place 250-cubic centimeter portions of the rain water sample in 500-cubic centimeter Erlenmyer flasks and add 5 cubic centimeters of Benedict's solution. Evaporate the solution to dryness on the hot plate in a 50-cubic centimeter evaporating dish, filling the dish each time that it is reduced about one-half. The last portion is evaporated carefully to avoid spattering. When thoroughly dry, place in the electric muffle and heat gradually to a red heat. The black residue will first fuse, then become dry, and all traces of nitrate and chlorates will disappear. Cool, take up with 10 to 20 cubic centimeters of dilute, 1 to 4, hydrochloric acid, warm gently until the contents of the dish have completely dissolved and a clear solution is obtained. Wash the solution into a small Erlenmyer flask, precipitate and determine the sulfur as described above.

REMARKS

The fusion should always be made with electrical apparatus. If such apparatus is not available special precautions must be observed to protect the fusion from sulfur contaminations in the gas. In this case use an alcohol blast lamp, or protect the top of the crucible with asbestos board, from contact with the flame as directed in the Determination of Potassium, page 151.

Barium sulfate precipitates in very fine crystals in the cold solution, and larger crystals in the hot solution. If the hot solution is kept in a warm place the crystals will grow in size.

In turbidity readings, where Na_2CO_3, Na_2O_2 or NaOH have been used and the solution made acid with HCl, considerable sodium chloride will be formed. The sodium chloride tends to promote the collodial character of precipitated $BaSO_4$. It is advisable to add approximately as much chemically pure NaCl to the standard turbidity solution as would be contained in the unknown solution. The addition is made before the barium chloride is added.

COMPLETE SOIL ANALYSIS

A complete soil analysis is made with one of two viewpoints. To determine

1. A total determination of all the elements contained in the sample.

2. A determination of all the elements in the sample that are capable of extraction by digesting with a strong acid. Such a series of determinations can only be regarded as a partial analysis of the elements present in the soil.

In the first case the results give no indication of the possibility of the elements becoming available for plant use. In the second case, an indication is secured that at some future time the elements may become available. The determinations differ only in the method of preparing the sample for analysis, *i.e.*, they bring the elements into solution. The soil sample is rarely so small that the determinations must follow each other in sequence. Furthermore, certain elements such as carbon, nitrogen, phosphorus, etc., may be determined more accurately in separate portions of the sample.

The Soil Sample.—Secure a generous amount of the soil sample as directed in Sec. 1, pages 13 to 15, break all large lumps with a wooden rolling pin, pass through a 6-mesh sieve (Bureau of Standards, 3.36 millimeter openings). Grind gently with a rubber-tipped pestle to break up all concretions, but avoid fracturing the pebbles. Pass through a 17.2-mesh sieve (Bureau of Standards sieve No. 8, 1 millimeter openings). In both cases the material not passing through is to be discarded. Grind in an agate mortar until the entire sample will pass a 100-mesh sieve (Bureau of Standards sieve No. 100) and store in stoppered bottles.

Moisture.—Determine moisture as directed under Hygroscopic Moisture, page 27, using 2 to 10 grams of the sample.

Volatile Matter.—The volatile matter is determined in separate portions of the soil sample (see Soil Alkali, page 92); Ammonia, page 96; Carbon, page 107; Nitrogen, page 126; Phosphorus, page, 140; and Sulfur, page 158).

Loss on Ignition.—Determine the loss on ignition as directed under Loss on Ignition, page 40. This determination is made upon the sample which is to be analyzed for its major constituents.

PREPARATION OF SOIL SOLUTION

Fusion with Sodium Carbonate.—Use the residue from the determination of the loss of ignition. Mix the ignited soil with five times its weight of pure sodium carbonate, place in a platinum crucible, cover the crucible and heat cautiously until the fusion is quiet. If the crucible is heated at first over a low flame, taking

care to avoid an oxidizing flame, or is heated in an electric furnace, there need be no trouble due to boiling over the edge. Continue the ignition for 10 minutes after the fusion is quiet, using the full height of the Bunsen or the blast. Remove the flame, sieze the crucible with platinum-tipped tongs and, while the contents are still liquid, rotate until the liquid cools in such a manner that very little of the material is on the bottom of the crucible. Gently roll the crucible between the fingers to loosen the solid material, which may be readily accomplished if the crucibles are kept smooth and well-scoured with sand. Disintegrate the fused mass in a platinum dish with hot water, add ½ to 1 cubic centimeter of alcohol to decolorize the manganate, and boil the mixture for 5 to 10 minutes.

Strong Acid Digestion.—Place the equivalent of 10 grams of air-dry soil in a 250-cubic centimeter Erlenmyer flask. Fit the flask with a reflux tube 20 inches or more in length. A special flask with a ground-glass joint may be secured, or a one-holed rubber stopper may be used. Add 100 cubic centimeters of hydrochloric acid of constant boiling point, specific gravity approximately 1.115 (1,350 cubic centimeters of hydrochloric acid, specific gravity 1.19 diluted with 1,000 cubic centimeters of distilled water). Digest on the steam or water bath for 10 consecutive hours, shaking the flasks every hour. Allow to settle, and avoiding more than small quantities of the sediment, decant the solution into a porcelain dish or non-soluble glass beaker. Transfer the insoluble residue, by means of hot water, to a washed filter, and wash free of chlorine and add the washings to the original solution. Concentrate this solution to approximately 10 cubic centimeters, oxidize any organic matter by the addition of 10 to 15 drops of concentrated nitric acid evaporating to dryness on the water bath. Treat with hot water and 2 or 3 cubic centimeters of hydrochloric acid and again evaporate to dryness. When the final evaporation is complete and the dish cool, moisten the residue with five drops of strong hydrochloric acid, add 10 to 20 cubic centimeters of distilled water to insure complete solution of the soluble salts, filter and wash free of chlorine. Again evaporate the solution to dryness to render any silica insoluble that may remain in solution, and treat as above. The filtrate consists of the soluble extract, freed of soluble silica. It is made up to a definite volume 250 or 500 cubic centimeters and labled.

Insoluble Residue.—Combine the filters and the main residue in a small dish; dry, ignite, carefully at first then strongly, and bring to a constant weight.

Determination of Silica:

When the solution from the sodium carbonate fusion is decolorized, and not before, carefully add 15 cubic centimeters of concentrated hydrochloric acid and evaporate the whole to dryness on the steam bath. The residue is digested on the steam bath with 15 cubic centimeters of concentrated hydrochloric acid and an equal volume of hot water, filtered, and washed free of chlorides. The filtrate is evaporated to dryness and heated for 2 hours at 105 to 110°C., taken up with 15 cubic centimeters of concentrated hydrochloric acid, filtered, and washed free of chlorides. The washings are combined and reserved for further determinations.

The second silica residue is combined with the first and both are ignited to constant weight slowly at first and then at a high temperature. Care is necessary to insure complete transfer of the silica to the filter paper and to prevent loss of silica from the crucible by air currents. The crucible should be kept at the temperature of the blast for at least 20 minutes, to insure complete dehydration. Bring the crucible to constant weight. The silica residue is treated, in the crucible, with 6 to 8 cubic centimeters of concentrated hydrofluoric acid and two to three drops of concentrated sulfuric acid. Care must be taken to see that all of the silica is brought in contact with the acid. Evaporate carefully to dryness. If the silica content is large, as is ordinarily the case with soils, the treatment with acid is repeated. The dry residue is ignited for a minute in the blast, cooled and weighed. The loss in weight represents the silica (SiO_2).

The contents of the crucible, if any, are taken up with a little dilute hydrochloric acid, washed out and added to the original soil solution.

REMARKS

It will be noticed that the soil solution, however obtained, is in a hydrochloric acid solution and that at this point all silica has been removed. Concentrate the solution to a definite volume, 250 to 500 cubic centimeters, and designate as "Solution A."

TREATMENT OF THE ACIDIFIED SOIL SOLUTION AFTER THE REMOVAL OF SILICA

Iron, Aluminum, Titanium, and Phosphorus.—A 50- to 100-cubic centimeter aliquot of the acidified soil solution (Solution A) is treated with ammonium hydroxide, drop by drop, until the precipitate formed requires several seconds to dissolve. The ammonium hydroxide must be free of all carbonates to prevent contamination of the precipitate by the alkaline earths. If an excess of ammonia is added the precipitate may be redissolved by the addition of hydrochloric acid. The reaction of the solution should be faintly acid. Heat nearly to the boiling point and add ammonium hydroxide, slightly in excess, to precipitate the iron, aluminum, phosphorus, and titanium. The precipitate is boiled in a covered beaker for a few minutes to expel the excess ammonia and to coagulate the gelatinous mass. A slight amount of ammonia must be present, and if no ammonia is given off (detected by smelling) add a small amount, drop by drop, until it can be detected. Do not allow the precipitate to settle, but stir and pour on the filter. Wash immediately with hot water, using a fine jet, playing it around the edge of the precipitate so as to cut it free from the edge, thus producing rapid filtration. Wash the precipitate several times and return it, with the filter paper, to the original beaker. Shred the paper with a stirring rod, make the volume up to approximately 150 cubic centimeters and dissolve with a few drops of hydrochloric acid. Filter out the paper, wash, warm the filtrate, and reprecipitate with ammonium hydroxide as above, washing with small quantities of hot water until no test for chlorides is obtained. Designate the filtrate and washings as "Solution B" and reserve for the determination of calcium and magnesium.

Dry the filter and precipitate, remove the latter from the filter, ignite the filter separately, and add to its ash the precipitate. Ignite to constant weight, slowly at first, then increase to a bright red. Weigh as ferric oxide (Fe_2O_3), aluminum oxide (Al_2O_3), phosphorus pentoxide (P_2O_5), and titanium dioxide (TiO_2). The titanium may be ignored.

If the residue from the silica determination was not added to the original solution, the ignition may take place in the crucible in which the silica was determined without removal of the residue. In this case the weight of the residue is combined with the above precipitate.

Determination of the Elements Precipitated by Ammonia:

Assuming That Titanium is Absent.—If the titanium is to be ignored proceed as follows: Transfer the ignited residue to a flask, digest with 5 to 10 cubic centimeters of dilute, 1 to 4, sulfuric acid, using heat. When solution is complete, reduce the iron with zinc and determine the ferrous iron by titration with a standard permanganate solution and calculate to ferric oxide (see Oxidation and Reduction, page 65). If desired the iron may be determined in an aliquot of the original solution, "Solution A," by adding an excess, 10 cubic centimeters, of sulfuric acid, or until all hydrochloric acid is expelled, diluting, reducing with zinc, and titrating with standard permanganate. The weight of the ferric oxide, plus that of the phosphorus pentoxid (which has been determined in a separate sample), subtracted from the total weight of the combined precipitates, gives the weight of the aluminum oxide.

Including the Determination of Titanium.—If the titanium is to be determined proceed as follows: Transfer the ignited precipitate to a large platinum crucible, add 5 to 7 grams of potassium pyrosulfate and heat gently over a Bunsen burner, keeping the crucible covered. A small portion of the pyrosulfate is added to the crucible in which the original ignition was made and melted to dissolve the adhering oxide. This melt is transferred to the large platinum crucible. The fusion must be carefully watched to avoid boiling over the sides. After the effervescence has subsided any adhering oxides above the melt must be brought into it by manipulation of the crucible with the tongs and flame. The cooled melt is dissolved in hot water to which 4 cubic centimeters of strong sulfuric acid have been added.

Determination of Iron:

Dilute the solution from the potassium pyrosulfate fusion to approximately 125 cubic centimeters in a 250-cubic centimeter beaker and pass hydrogen sulfide gas into it for about 5 minutes to reduce the iron and precipitate any dissolved platinum. Boil to coagulate the sulfur and filter into a 300-cubic centimeter Erlenmyer flask. The flask is fitted with a two-holed rubber stopper provided with an inlet tube leading to the bottom of the flask, and an outlet tube with the opening flush with the bottom of the stopper. The solution is diluted to approximately 200 cubic centimeters and hydrogen sulfide gas passed through.

While the gas is passing through, bring the solution gradually to the boiling point and cool for 10 minutes. Stop the flow of gas and expel the excess hydrogen sulfide by running a stream of carbon-dioxide gas through the hot solution in the flask until a strip of filter paper, moistened with lead acetate solution and held at the outlet tube, fails to give a test for hydrogen sulfide. The solution is now cooled without interruption of the current of carbon-dioxide gas. When the solution is cold it is titrated at once with standard potassium permanganate, introduced through the inlet tube. The result is checked by repeating the reduction and titration. For details consult, United States Geological Survey *Bull.* 422; 107–109, 1916, or United States Geological Survey *Bull.* 700; 119–121, 1919.

Determination of Titanium:

The solution from the iron titration is evaporated to 50 or 75 cubic centimeters. Add 10 cubic centimeters of concentrated sulfuric acid and 1 to 2 cubic centimeters of hydrogen peroxide. The color produced is compared with that of a standard potassium titanium fluoride (K_2TiF_6) solution, produced in a like manner. The comparison is made according to ordinary colorimetric processes.

ALUMINUM

The per cent of aluminum Al_2O_3 is calculated from the total weight of the original precipitate after deduction is made for the iron (Fe_2O_3), phosphorus (P_2O_5), and titanium (TiO_2).

TREATMENT OF THE SOIL SOLUTION AFTER THE REMOVAL OF ELEMENTS PRECIPITATED BY AMMONIA

Solution B.— Combine the filtrates and evaporate to about 100 cubic centimeters. Transfer to an Erlenmyer flask and add a drop of hydrochloric acid. Add about 5 cubic centimeters of ammonium sulfide, or 3 cubic centimeters of ammonium hydroxide, and pass hydrogen sulfide in until the solution is saturated. Now add 2 to 3 cubic centimeters of ammonium hydroxide, stopper the flask tightly, and allow to stand overnight. The precipitate is filtered and washed with ammoniacal ammonium sulfide. The precipitate is a mixture of the sulfides of zinc, copper, manganese, etc., and need not be examined further unless an estimation of manganese is desired, in which case, proceed as

directed under Determination of Manganese, below. The filtrate contains calcium and magnesium.

CALCIUM

The hot filtrate from the ammonium sulfide precipitation is treated with an excess of recently dissolved ammonium oxalate and digested for ½ hour on the water bath. The cooled solution is filtered and the precipitate washed and ignited. The ignited residue is redissolved in dilute hydrochloric acid, reprecipitated, ignited and weighed (see Determination of Calcium), page 105.

MAGNESIUM

The filtrates and washings from the calcium determination are combined and the magnesium determined in the usual manner (see Determination of Magnesium), page 124.

Determination of Manganese:

Concentrate an aliquot of Solution A to about 50 cubic centimeters; cool; add bromine water until the solution is colored; make alkaline with ammonium hydroxide, and heat to boiling in a covered beaker; cool; repeat the addition of bromine and ammonia. If a precipitate is obtained, slightly acidify the solution with acetic acid, filter immediately and wash with hot water. Dry the precipitate, ignite and weigh, as manganomanganic oxide (Mn_3O_4).

REMARKS

The elements carbon, both organic and inorganic, manganese, nitrogen, potassium, sodium, sulfur, and usually phosphorus are determined in separate samples, using the methods previously described. Ammonia and chlorine are ordinarily not determined. When they are, the ammonia is determined as directed under Determination of Ammonia, page 97, and chlorine as directed under Soil Alkali, page 93.

Report results as follows:

Insoluble residue	Soluble silica
Mn_3O_4	K_2O
Na_2O	CaO
MgO	Fe_2O_3
Al_2O_3	P_2O_5
Organic carbon	Inorganic carbon
Sulfur trioxide	Nitrogen
Ammonia	Chlorine
Lime requirement	Volatile matter

Other soil compounds as barium, chromium, zirconium, etc., are usually present in such small amounts as to render their determination unnecessary. If for any reason it is desired to make a determination of these elements, the analyst is referred to *United States Geological Survey Bulletin* 700 as a guide, supplemented by the suggestions in *Bureau of Soils Bulletin* 122, and in the *Publications of the Association of Official Agricultural Chemists.*

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SECTION 4

SOIL MICROBIOLOGY

Soil Microbiology may be defined broadly as a study of the life history and characteristics of each form of microscopic life found in the soil. The methods used are those of the bacteriologist, mycologist, protozoölogist, etc. The soil investigator is interested primarily in those microbiological activities that affect soil fertility, consequently he studies the complex microscopic life from the standpoint of its influence on the availability of plant food. He uses mainly the same methods used in the Fertility Determinations, namely those of the chemist.

The activities of the microorganisms in the soil are governed by the same factors that govern plant life, namely, food, moisture, temperature, air, biological relations, and reaction. Practically all of the microorganisms are dependent upon some form of carbon as food. In decomposing and assimilating the carbohydrate portion of the molecule, the mineral portion is usually split off as a waste product. The mineral elements or compounds, rejected by one group of microorganisms, may serve as a direct food supply for the higher plants, or they may be acted upon by other microorganisms and brought into a suitable condition for plant use. The CO_2 produced by the microorganism as a waste product plays a very important role in bringing into solution the insoluble constituents of the soil.

The relations existing between the higher plants and microorganisms are *inseparable*. The higher plants are mainly dependent upon the activities of the microscopic soil life for the major portion of their soluble minerals, while the latter are equally as dependent upon the former for their supply of carbon.

THE MICROSCOPIC LIFE IN SOILS

The statement has been made that, "Given the proper time, the proper place, and the proper cultural method, every form of microscopic life may be found in the soil." While this is possible, we are only interested in those forms that habitually live in the soil and affect its fertility.

The Soil Sample.—The samples for biological studies are collected from a number of places in the selected area, similar to the method followed in collecting the samples for physical and fertility determinations. The only difference in collecting samples for biological study, is that all of the equipment used must be bacteriologically clean (sterile). All containers, tools, etc., should be sterilized in the autoclave before taking them to the field. If the same equipment is to be used for taking a number of samples, some method must be arranged for field sterilization. A very satisfactory procedure is to wipe all equipment with a cloth saturated with 50 per cent alcohol and allow to dry each time that a new area is to be sampled. Pint or quart Mason jars make the most satisfactory containers. Great care must be exercised to prevent the contamination of one sample with the organisms contained in another. This point cannot be made too emphatic, especially when soils from different depths are being studied. The exact method to be followed is left to the discretion of the investigator.

It must be remembered that as soon as a soil is disturbed the increased air supply may cause conditions that will allow the microorganisms, particularly the bacteria, to increase in numbers. Under very favorable conditions, marked increases may take place within 30 minutes, consequently the sample must be taken to the laboratory and studied at once.

QUALITATIVE STUDIES

An idea of the complexity of the soil population may be secured by a microscopic examination.

Preliminary Examination of a Soil.—Place several loopfuls of sterile, distilled water on a clean slide. A small portion of the soil to be examined is stirred by means of a stiff wire loop, into the water and the whole spread into a thin film, so that one can readily see between the particles. Cover with a cover-glass and examine by means of the low-power objective. Note the presence of any moving objects. Nematodes and the protozoal forms may be readily observed by this process. The protozoa and amœbae may be stained by the use of a drop of 0.5 per cent aqueous solution of neutral red. The drop is placed on one side of the cover-glass and drawn through with a piece of filter paper touching the other side.

As the above forms do not occur in large numbers in field soils, special soils must be used in order to become acquainted with them. Protozoa and amœbae may be found in soils secured from a low, wet area, or from a soil that has been kept at about three-fourths saturation, with protozoa solution for 3 weeks. Nematodes usually may be found in any greenhouse soil that has been in place for several years. If a root, infected by these organisms, is dried slowly, it will make excellent laboratory material. The slow drying causes the worms to encyst. If the dried material is placed in water for 2 or more hours, the worms emerge from the cysts. In case the organisms are very active and the observer is unable to follow them, a few threads of absorbent cotton, placed criss-cross in the solution, will retard their movements. If the observer wishes to keep the soil under observation for long periods, the use of the Sedgwick-Rafter cell, as used in the counting of protozoa, is recommended.

Preliminary Examination of a Soil Solution.—In a test tube prepare an extract of different kinds of soil and of several samples of manure, by shaking 2 to 5 grams in twice the volume of water. Place one or two drops of the solution on a clean slide and cover with a cover-glass, examine with the low-power and the high-power dry objectives. Make a hanging drop and examine under the oil immersion. Note the different forms. Distinguish if possible, the presence of fungi, algae, diatoms, amœbae, protozoa, and bacteria. Add a drop of neutral red to the solution and note which of the organisms take the stain. Dry the solution on the slide and make stained preparations using aqueous fuchsine, methylene blue, and iodine as stains. Compare the size, number, and character of the different forms found in the different soils with those found in the manure.

Spore-forming Bacteria in the Soil.—Some of the bacteria in the soil are in an inactive (spore) state under normal conditions. The number, in the spore form, may be determined by comparing the total counts of pasteurized and unpasteurized soils.[13] As this method is tedious and gives no indication of the activity of the organisms, it is suggested that the presence of the spore forms be first determined qualitatively by a microscopic examination.

Microscopic Examination of a Soil for Spore-forming Bacteria. Prepare a fixing solution of 0.2 gram of gelatin in 500 cubic centimeters of water. Sterilize in the autoclave. Place ½ to 1 gram of soil in a test tube and add 6 to 8 cubic centimeters

of the fixing solution and shake thoroughly for 2 minutes. Remove two loopfuls of the solution, place on a clean slide, and make a smear of them. Allow the smear to dry and stain with a 1 per cent solution of rose bengal in a 5 per cent phenol-water mixture. A microscopic examination of the smears will show that the non-spore formers of the soil are under 0.8 micron in diameter, while the vegetative forms of the spore formers are nearly always over 1 micron.

THE NUMBER OF SOIL ORGANISMS

The total number of microorganisms commonly living in the soil is very large and represents many complex forms. The largest group, from the standpoint of numbers and possibly activities, is undoubtedly the bacteria. This group may be equaled, if not excelled in some cases, by the number of thread bacteria (actinomycetes), the filamentous fungi, or by the protozoa. Many other forms as algae, yeasts, etc., are always present. As the requirements for the growth of each form are entirely different, it is necesary to separate the microorganisms from the soil particles and then place them in a medium favorable for their development. The separation of the organisms from the soil is usually accomplished by diluting a weighed amount of the soil in a measured amount of water. A dilution made in this manner is called a "Soil Infusion."

Preparation of a Soil Infusion.—One hundred grams of fresh, moist soil is introduced into 200 cubic centimeters of sterile tap water and thoroughly shaken for 10 to 20 minutes. The larger particles of soil are allowed to settle out and practically all of the organisms will be in the suspension. The number of microorganisms is determined by making dilutions as outlined below. If counts are to be made the dilutions must be made and the plates poured within 30 minutes. If the infusion is to be used for the purpose of inoculating other soil, the time may be extended to 3 hours. Infusions over 3 hours old are of questionable value.

Diluting the Soil Infusion. The number of organisms present is determined by accurately diluting, in definite amounts of sterile tap water, the soil infusion, until a point is reached where the investigator will feel sure that only a small portion of the original number are present. It is important, however, that this number is a true representative of the total number present

in the original soil sample. The amount of dilution varies according to the results desired. In all cases the amount withdrawn from the infusion should not be less than 10 cubic centimeters. The following table indicates the procedure to follow in making dilutions. Designate the infusion as dilution A, each cubic centimeter of which contains, in suspension, the organisms originally present in ½ gram of soil.

TABLE 5.—DILUTION OF SOIL INFUSION

Place.....	In sterile tap water	Each cubic centimeter is equivalent to	Dilution	Designate as dilution
10 cubic centimeters A	490 cubic centimeters	0.01 grams	1/100	B
10 cubic centimeters B	90 cubic centimeters	0.001 grams	1/1,000	C
10 cubic centimeters C	90 cubic centimeters	0.0001 grams	1/10,000	D
10 cubic centimeters D	90 cubic centimeters	0.00001 grams	1/100,000	E

Each dilution is made with a sterile pipette which is discarded after being used. Each dilution is thoroughly shaken after inoculation. In case dilutions are being made from solutions containing clumps or masses of organisms, these clumps may be broken up by first inoculating 10 cubic centimeters of the infusion into 90 cubic centimeters of sterile water containing approximately 10 grams of clean sand and shaking thoroughly.

Determination of the Number of Bacteria:

The number of bacteria in soils, water, manures, etc., is determined by placing 1 cubic centimeter of each dilution into a separate Petri dish and adding approximately 10 cubic centimeters of a sterile agar medium, cooled to 40°C. The agar is quickly poured under the partially raised lid and the plate tilted and gently rotated to insure uniform distribution of the organisms. All plates must be poured in duplicate, preferably in triplicate, while for more exact work a larger number of duplicates must be used.

The number of dilutions to be used must be determined experimentally. If the approximate number of bacteria is not known

the dilutions should extend from $\frac{1}{1,000}$ to $\frac{1}{10,000,000}$. For routine work the number may be reduced, but in no case should less than two dilutions be used. The ideal dilution is one that will give a growth of 50 to 200 bacterial colonies per plate.

The Medium.—The medium to be used depends upon a number of factors. Some workers apparently get better results with one formula than with another, while others may use two or more for the determination of the same group of organisms. The following are recommended:[18, 3]

1. For Total Numbers of Bacteria and Actinomycetes:

(a) Sodium Albuminate Agar

Distilled water	1,000 cubic centimeters
Dextrose	1.0 gram
Di-potassium phosphate (K_2HPO_4)	0.5 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 gram
Ferric sulfate ($Fe_2(SO_4)_3$)	trace
Egg albumin	0.25 gram
(The egg albumin is dissolved in $\frac{N}{10}$ NaOH and made alkaline to phenolphthalein.)	
Agar	15.0 grams

(b) Sodium Caseinate Agar

Made the same as the sodium albuminate agar, except that 1 gram of casein (dissolved in 8 cubic centimeters of $\frac{N}{10}$ NaOH) is substituted for the egg albumin. The casein should be made according to Hammarsten or Van Slyke method. If nutrose (sodium caseinate) is used, no neutralization is necessary.

(c) Soil Extract Agar or Gelatin

Soil extract	100 cubic centimeters
Distilled water	900 cubic centimeters
Di-potassium phosphate (K_2HPO_4)	0.5 gram
Agar	15.0 gram
or	
Gelatin (gold label)	150 grams

If desired 5 grams of sodium chloride may be added to prevent the spread of liquifying colonies in the gelatin.

The reaction of this medium should be carefully adjusted to a P_H of 6.8 (see, Colorimetric Determination of Hydrogen-ion

Concentration, page 70.) During sterilization the medium may become more acid. After the final sterilization the hydrogen-ion should be about 6.5.

Soil Extract.—Heat 1 kilogram of rich soil with 1 liter of tap water for 30 minutes, in the autoclave at 15 pounds. Decant the turbid liquid, add a litle talc, and filter through a double, folded filter. Obtain approximately 800 cubic centimeters of the filtrate.

2. For Total Numbers of Nitrogen Fixers (Azotobacter):

Nitrogen-free Dextrose Agar[7]

Distilled water	1,000 cubic centimeters
Di-potassium phosphate (K_2HPO_4)	0.2 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 gram
Calcium chloride ($CaCl_2$) fused	0.02 gram
Dextrose	10.0 grams
10 per cent ferric chloride solution	2 drops
Agar	15.0 grams

Dissolve the salts, heat the solution to boiling, and make neutral to phenolphthalein by the addition of $\frac{N}{10}$ NaOH. Add the sugar of the agar, sterilize at 10 pounds for 20 minutes. A greater pressure will result in carmelizing the sugar. Saccharose or mannite may be substituted for the dextrose.

Incubating the Plates.—The agar plates are incubated at a temperature of 28 to 30°C. or, if the room temperature is fairly constant during the 24-hour period, they may be incubated in some protected position (locker) in the laboratory. Gelatin plates are always incubated in the refrigerator or in a low temperature incubator. The incubation temperature is always noted.

Determination of the Various Forms Appearing on the Plates: Usually the plates will be contaminated with a few fungi, while actinomycetes will be found in all of them. These are not strictly contaminations, rather they are forms that are capable of growth on the particular medium. While the student will be able to distinguish the different colonies on the old plates, he cannot do this during the earlier stages of growth until he has become more experienced. He can, however, readily distinguish the various forms by making a microscopic examination of the colonies. This is accomplished as follows: Invert the plate and

place upon the stage of the microscope. Focus the low-power adjustment upon the edge of the colony to be examined. The edge of the bacterial colonies will be smooth and definite. The line will not always be a part of a circle, it may be wavy, lobed, thick or thin, but the edge is always smooth and well defined. The edge of the fungi is always thready, the threads growing in all directions from a common center. In some cases it may be difficult to find the edge, the colony appearing as a mass of hyaline threads. The edge of an actinomycete colony is always definite but never smooth. Under the microscope it appears as a mass of short, interlocking threads. The thin edge, so common in the bacteria, is absent.

Counting the Plates.—The plates are examined and counted on the third, fifth, seventh, and tenth days. The actinomycetes are allowed to grow for 14 days before the final count is taken. The final count is made by means of the counting plate (Jeffer's). The earlier counts are made by checking the position of each colony with a glass pencil, the mark being made on the bottom of the plate. The earlier counts are for the determination of the quick growing forms, which in the lower dilutions quickly crowd the plates; the later counts include the slower developing forms, hence give a more accurate determination of the total number.

Expression of Results.—Results are always expressed on the air-dry basis, *i.e.*, number of organisms per gram of air-dry soil. It is necessary, therefore, to weigh out in duplicate, samples of the moist soil at the time the infusion is made and determine the per cent of moisture (see, Moisture, Sec. 2, page 26). The adjustment, from the moist to the air-dry basis is made as follows: Assume that the moisture content is found to be 17 per cent and that the most satisfactory count is found in dilution D, representing $\frac{1}{10,000}$ gram of moist soil. The average count on these plates is assumed to be 163 colonies, thus representing 1,630,000 organisms in 1 gram of the moist soil. We have found that only 83 per cent of the total soil sample has become air-dry, therefore, $\frac{1,630,000}{83} \times 100$ would equal the number of organisms present in 1 gram of the air-dry soil—in this case 2,000,000 organisms.

Suggested Experiment

It may be assumed that 500,000,000 bacteria weigh 1 milligram and that 80 per cent of this weight is moisture. Also that 100,000,000 bacteria occupy a space equivalent to 0.2 cubic millimeter. The analysis of bacteria

on the dry basis, shows that they contain approximately 2.3 per cent nitrogen and 1.2 per cent phosphorus. Calculate the weight and space occupied by the bacteria in an acre of soil to a depth of 6⅔ inches. Calculate the amount of nitrogen and phosphorus contained in the bacterial cells.

Determination of the Number of Filamentous Fungi:

The number of filamentous fungi in the soil is determined in exactly the same manner as recommended for the determination of the bacteria, using a medium that is acid in reaction and contains a large amount of soluble carbohydrate. Such a medium is also favorable for the growth of yeast colonies as well as certain bacteria that apparently prefer this type of medium. It is usually quite difficult for the inexperienced worker to differentiate between the yeast and bacterial colonies unless bacteriological mounts are made and examined under the microscope. Until such times as one is able to distinguish between the two forms, both are reported as bacterial colonies. The total number of fungi in the soil is usually one-tenth to one-fiftieth that of the total number of bacteria. For field soils the dilutions are prepared and the plates poured accordingly. If it is desirable to determine both bacteria and fungi in the same infusion one merely pours a greater number of plates and uses a wider range of dilutions. The lower dilutions are used for making counts of the fungi, the higher for the bacteria and intermediate dilutions are for both forms. For instance, in the dilutions suggested, the B, C, and D plates would be poured for the determination of fungi, using the proper medium, while the C, D, and E plates would be poured for the determination of the bacteria and actinomycetes, using a medium for their best growth.

Media for the Determination of Soil Fungi:

The media presented below[12 and 18] is designed to favor the growth of the filamentous fungi. No satisfactory technique has been developed for culturing the fleshy fungi of the soil.

(a) Soil Extract Agar

Distilled water	800 cubic centimeters
Soil extract	200 cubic centimeters
Dextrose	10 grams
Di-potassium phosphate (K_2HPO_4)	0.5 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 gram
Agar	15 grams

The soil extract is prepared as described under Media for Bacteria.

(*b*) *Synthetic Acid Medium*

Distilled water	1,000 cubic centimeters
Dextrose	10 grams
Peptone	5 grams
Mono-potassium phosphate (KH_2PO_4)	1 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.5 gram
Agar	25 grams

Dissolve the sugar, peptone, and salts in the water, add hydrochloric acid to adjust the P_H to 4.0, add the agar and sterilize.

Period of Incubation.—The period of incubation is from 3 to 7 days at the same temperatures recommended for the bacteria.

Active Soil Fungi.—If one wishes to determine whether or not the fungi in the soil are in an active (vegetative) state, the procedure is as follows: Pour sterile plates of agar and inoculate with chunks of soil about the size of a small pea. Press the soil into the agar being careful not to break apart the particles. Make a spore suspension of fruiting fungi and inoculate marked areas of other sterile plates with the suspension. Examine at the end of 12, 15, 18 and 24 hours. If the fungi are in an active state, a fine mycellia will be seen growing out from the edges of the soil masses before growth is apparent from the spores.

Active Fungi in Manure.—The presence of active fungi in manure may be demonstrated in the same manner as recommended for soils. The growth of forms, not developing on plates, may be demonstrated by placing fresh horse dung under a Bell jar and keeping it moist and warm. If the jar is partially lined with wet filter paper and kept in subdued light, the *Pilobolus* and *Pilaria* that develop will discharge their spores toward the unlined side.

REPORTS

The total number of bacteria, actinomycetes, and fungi may be reported on the same blanks. A suggested heading for such a blank is given below.

NUMBER OF MICROORGANISMS FOUND IN SOILS

1. Soil source	2. Medium	3. Dilution	4. Incubation period

Plate counts			Organisms per gram moist soil		
5. Bacteria	6. Actinomycetes	7. Fungi	8. Bacteria	9. Actinomycetes	10. Fungi

11. Per cent of moisture	Organisms per gram air-dry soil		
	12. Bacteria	13. Actinomycetes	14. Fungi

15. Ratio of bacteria, to actinomycetes, to fungi

Determination of the Number of Protozoa:

The protozoan life in soils is usually of two forms, active and inactive (encysted). The total number is usually less than that of the fungi. The following method[5] is designed to determine both forms. It is based upon the fact that the active forms are killed in a 2 per cent hydrochloric acid solution while the inactive (encysted) forms are not affected. This requires that two determinations be run on duplicate soil samples. The first is for the determination of the active forms and the second for the determination of the encysted forms.

Weigh out two 10-gram portions of soil. Portion 1 is to be diluted at once as directed below. Portion 2 is treated with hydrochloric acid of such strength that all carbonates are neutralized and still leave a strength of 2 per cent. This infusion is allowed to stand overnight. The dilutions are made in sterile tap water or in a physiological salt solution (0.85 per cent) as follows: 1 to 10; 1 to 100; 1 to 1,000; 1 to 2,500; 1 to 5,000; 1 to 7,500 and 1 to 10,000. These dilutions will suffice for field soils, but higher dilutions will be necessary for greenhouse soils.

Pour approximately 15 cubic centimeters of sterile, nutrient agar (any agar suggested for bacterial determinations) into sterile Petri dishes and allow to harden. Inoculate the plates in duplicate by allowing 1 cubic centimeter of a dilution to flow over the surface of the agar. Incubate in a moist atmosphere for 28 days. Examine the plates under the low power for the presence of pro-

tozoa at 7-day intervals. The highest dilution giving growth is taken as an indication of the number of organisms. Note should be made of the different forms found growing in the different dilutions.

Some investigators find that the use of hay infusion in tubes, instead of agar plates, gives quicker results. Such results are not accurate, due to the fact that many of the forms need large amounts of oxygen and will not develop in a liquid medium. If a liquid medium is desired, satisfactory results may be secured by the use of sand slants.

Sand Slants.—The slants are prepared by introducing into a 250-cubic centimeter Erlenmyer flask, containing 50 to 75 cubic centimeters of a nutrient solution favorable to the growth of protozoa, a sufficient amount of clean sand to maintain an exposed portion in the presence of the nutrient solution. The flasks are tilted to one side after sterilization to allow the sand to pile up. When they are replaced in their original position a portion of the sand will remain exposed. Inoculation is made in the usual manner. The flasks are incubated at 25 to 30°C. for 30 days and are examined every seventh day. The protozoal growth will be found in the various levels of the sand and solution, consequently it is important that the slope of the slant be maintained as far as practicable.

Direct Examination of the Number of Protozoa.—If the presence of protozoa is suspected but the dilution is too great, or it is desired to examine the active forms directly, the following is recommended:

Mix 100 grams of fresh moist soil with 500 cubic centimeters of sterile tap water in a large crystallizing dish. It must be remembered that the protozoan forms are very fragile and easily crushed, hence all operations must be conducted as gently as possible. Allow the soil to settle and decant the water. Repeat the washing, decant, and add to the original. The majority of the protozoa are now free of the soil but are diluted in approximately 900 cubic centimeters of water, therefore must be concentrated.

Concentration of a Dilute Solution of Protozoa.—Fit a 35-cubic centimeter crucible with a disc of coarse filter paper and place on top a layer of clean sand, connect with a suction flask, and apply gentle suction. The protozoa are concentrated upon the sand and paper. When filtration is complete, wash the sand and paper into a small beaker with 10 cubic centimeters of sterile

water. Each cubic centimeter of this dilution represents the protozoal content of 10 grams of soil.

Automatic Filter.—If a number of soils are to be examined, time will be saved by having the filters automatic in action. Transfer the diluted soil solution to a 1,000-cubic centimeter Florence flask and fit the flask with a two-holed rubber stopper containing 2 glass tubes, 2 and 6 inches long, respectively. The long tube projects about ¾ inch above the top of the stopper, the short tube about 1 inch. The flask is inverted directly over the Gooch and clamped in such a position that both tubes will project into it. The solution will flow through the free end of the short tube until the air supply is cut off by it, automatically sealing the end of the long tube.

Counting the Soil Protozoa.—Thoroughly clean a Sedgwick-Rafter cell.[11] (This cell is a bacteriological slide 25 by 75 millimeters, to which is cemented a plate 1 millimeter thick, containing a rectangular opening 20 × 50 millimeters, the cubic content of which is equivalent to exactly 1 cubic centimeter.) By means of a bacteriological loop, thoroughly cover the bottom of the cell with a thin layer of phenolsulphonephthalein solution. (This material is prepared in ampules for injection in the renal efficiency test.) The solution is used without altering its composition and allowed to dry.

Dilute exactly 1 cubic centimeter of the solution containing the protozoa, with 1 cubic centimeter of 4 per cent gelatin, which has been neutralized with $\frac{N}{5}$ sodium hydroxide, using methyl red as an indicator. Fill the cell with exactly 1 cubic centimeter of this mixture, cover with a cover-glass and count under low-power objective, using the mechanical stage.

Suggested Experiments

1. Select areas of the same type that have received various treatments, lime, manure, green manure, fertilizers, etc., and determine the effects of these treatments on the total number of soil organisms. If treated soils are not available, make additions of these materials to a measured quantity of soil, 1 to 2 quarts, and determine the numbers at stated intervals, 1 to 4 weeks.

2. Vary the moisture content in the above, using 10, 20, 30, etc., per cent of saturation and determining the numbers at stated periods.

3. Compare the micro-biological content of cultivated and non-cultivated soils, either at a stated time or at various seasons of the year.

4. Determine the ratio of bacteria, actinomycetes, and fungi in field and wooded soils.

5. Determine the seasonal variation in numbers and the relationship of the various forms, by taking samples from the same area at monthly intervals.

6. Determine the influence of roots by taking the sample close to the roots of a vigorous plant and at a distance where no roots occur.

PURE CULTURE STUDIES

The soil investigator is usually interested in the activities of the microorganisms as a whole, consequently he refers the study of individual forms to the soil bacteriologist, mycologist, protozoologist, or the botanist. The study of each group of organisms is highly specialized. Each subject occupies an entire, and very fertile field in itself, therefore the investigator who wishes to enlarge his studies in these directions is urged to consult those sources of information dealing with the special forms under observation.

The isolation of special groups of particular interest will be discussed under their respective headings, for instance, the isolation for pure culture study of the ammonia oxidizing organisms is described under The Biological Oxidation of Ammonia.

In general, these forms, bacteria, yeasts, actinomycetes and fungi, appearing on the agar plates, may be isolated in pure culture by means of a sterile needle. A portion of a well-isolated colony, or the entire colony, is transferred to a tube containing the same medium, using all bacteriological precautions. For further study, the investigator is referred to the Chart of the Society of American Bacteriologists, and the voluminous literature on the different subjects.

The separation of protozoa from bacteria is difficult, while their culture is still more difficult.*

CHEMICAL STUDIES OF THE ACTIVITIES OF MICROORGANISMS

As indicated, in the beginning of this section, the basis of microbiological soil studies is the measurement of the activities of the entire fauna and flora in a unit of soil. The amount of any one of the plant food elements in the soil is usually very small, and the result of one group of microbiological activity may be

*The following references are given as a guide: *Soil Science* 2, No. 2, p. 163, 1916: *Science, N. S.* 43, No. 1098, p. 68, 1916: *Jour. Agr. Sci.* 7, Pt. 1, p. 106, 1915.

utilized or transformed by another group before a sufficient amount has been changed to render a chemical measurement possible. Consequently, the activities of certain groups are stimulated by the addition of some medium that will produce the desired results in a certain length of time (incubation period). Whenever possible, the soil should always be used in the fresh state, at least within a few hours after taking the sample. However, it is not always convenient to use fresh soil samples. There is a marked variation in samples taken from different parts of a field and at different times. Furthermore, large samples for class use cannot always be secured conveniently. Bacteriological studies of greenhouse soils would require the withdrawal of prohibitive amounts of soil, especially if the experiments were carried out in pots. These difficulties may be satisfactorily overcome, by recognizing that the efficiency of a soil is dependent upon the chemical and biological factors contained within it, and modifying the procedure accordingly.

The Chemical Factor in Soils.—It is a well-known fact that the elements in a soil do not change their combinations if the soil is kept in an air-dry state. Consequently, if representative samples of the area to be tested are secured, dried, and thoroughly mixed, the question of chemical differences, due to sampling, will in a great measure be overcome. The biological factor in an air-dry soil is not removed, the greater portion of it being in an inactive state.

The Biological Factor in Soils.—If a sample of air-dry soil is moistened and placed under favorable temperature conditions, it will soon be teeming with microscopic life. However, some of the forms originally present have been killed by the drying, while others emerge slowly from the spore or encysted state, thus giving the quick developing forms a chance to establish themselves. In other words, the sample will have an unbalanced biological relationship. If a sample of air-dry soil is inoculated with an infusion of fresh soil and the moisture content adjusted to the optimum, the biological factor is, for all practical purposes, quickly reestablished in approximately its original form. Advantage may be taken of this for both class and investigational work. A large amount of soil may be stored, thus insuring the same chemical factor. Each time that the biological factor is to be introduced the area under investigation must be visited and fresh samples secured.

Soil Sterilization.—The soil itself is the best culture medium for the growth of those biological forms that habitually live in it. As one often desires to determine the functions of certain pure cultures in the soil, it frequently becomes necessary to sterilize it. This is a difficult procedure and no definite recommendations can be given for its accomplishment. The only sure method is to autoclave the soil until plate cultures show a negative result. Usually this procedure produes marked changes in both the chemical and physical properties of the soil. Many methods have been suggested to overcome this difficulty. A marked improvement in the technique has been made, but no absolute method has been devised. It is claimed that the bacterial and protozoan life in moist soils can be almost completely decimated by heating at a temperature of 82°C. for 5 days (intermittent sterilization), without producing any marked change in the chemical constituents.

Biological Production of Ammonia.—The nitrogen in organic matter is always in combination with carbon compounds. The microorganisms need the carbon, for their metabolic processes, more than they need the nitrogen. As a result of their activities, in decomposing the protein molecule, the amid (NH) and the amino (NH_2) groups are hydrolized and split off in the form of ammonia. The action of the urea bacteria may be used as an example.

$$(NH_2)_2CO + 2H_2O = 2NH_3 + H_2O + CO_2.$$

As the ammonia may be easily determined, this process serves as a quick and effective means of measuring a phase of biological activities.

Determination of Ammonia Production in Solution:

1. Make a paste of 10 grams of Difco peptone with distilled or tap water and dissolve in a total of 1,000 cubic centimeters of water. Measure out 100-cubic centimeter portions and place in 250-cubic centimeter Erlenmyer flasks. Plug and sterilize at 15 pounds for 15 minutes.

2. Inoculate in duplicate with 10-cubic centimeter portions of fresh soil or manure infusions, and incubate 3 to 4 days at room temperature.

3. At the end of the incubation period, determine the amount of ammonia formed as recommended for ammonia determination. (See Ammonia, Sec. 3, page 98.)

If not convenient to complete the determination at the end of the incubation period, the action may be stopped by the addition of 10 cubic centimeters of 10 per cent copper sulfate solution.

Isolation of Ammonia Forming Organisms.—The causative organisms may be isolated in pure culture by diluting a few loopfuls of the growth in the peptone, in approximately 10 cubic centimeters of sterile water. From this dilution inoculate tubes of sterile bouillon agar, pour plates and pick off the isolated colonies. Dilute, replate, and transfer the growths obtained, until their purity is assured, and test for their ammonia producing powers in the above peptone solution. As many bacteria, and practically all fungi and protozoa, have the power of liberating ammonia from organic nitrogenous compounds or materials, this phenomenon is regarded merely as one of the cultural characteristics of the microorganisms.

Isolation of Urea Bacteria.—Urea decomposing bacteria may be readily isolated by inoculating urea solution with soil or horse manure, testing for ammonia production and isolating on urea agar.

Löhnis Urea Solution

Urea ($CO(NH_2)_2$)	50 grams
Di-potassium phosphate (K_2HPO_4)	0.5 gram
Soil extract	100 cubic centimeters
Tap water	900 cubic centimeters

The soil extract is prepared as described under Media for Bacteria, page 179. If a solid medium is desired add 15 to 20 grams of agar.

Determination of Ammonia Production in Soil:

1. Sieve, thoroughly mix, and determine the optimum moisture content of the soils to be studied.

2. Weigh out in tumblers, six 100-gram portions of the soils to be studied. If the soil is not air-dry, the equivalent of 100 grams of air-dry soil is to be used. Make additions as follows:

Tumblers	Additions
1 and 2	Nothing (check).
3 and 4	5 grams of dried blood (sieved).
5 and 6	5 grams of cotton seed meal.

3. Stir in these materials thoroughly by means of a sterile spatula.

4. If air-dry soil has been used, inoculate each kind of soil with 10 cubic centimeters of the corresponding infusion.

5. Adjust the moisture content to optimum with sterile water. If moist soil has been used, the amount of water in it must be determined and compensated for.

6. Add 12 cubic centimeters of water additional to those tumblers containing the dried blood and cottonseed meal in order to compensate for their absorbing effects.

7. Cover the tumblers with metal covers to prevent evaporation, and incubate 5 to 7 days at room temperature.

8. Determine the amount of ammonia produced as directed under Ammonia, Sec. 3, page 98.

9. Make reports on blanks headed as follows:

Biological Production of Ammonia

1. Soil source	2. Treatment	3. Medium	4. Dilution

Titration

5. Cubic centimeters $\frac{N}{10}$ acid used in receiving flask	6. Cubic centimeters $\frac{N}{10}$ alkali required to neutralize	7. Difference

8. Milligrams of N as ammonia found	9. Milligrams of N formed from the nitrogenous materials

10. Milligrams of N added in organic matter	11. Per cent of N in nitrogenous material reduced to ammonia

Suggested Experiments

The following factors are of importance and should be determined if time permits. The "Ammonia Production in Soils" above is to serve as a basis for making the following experiments:

1. Determine the influence of moisture by varying the moisture content as follows: 4 per cent, 8 per cent, 12 per cent, etc., up to 30 per cent or higher.

2. Compare the influence of air drying the soil, adjusting to optimum with sterile water (omitting inoculating with an infusion), with the same soil in the fresh moist condition.

3. Determine the influence of soluble carbohydrates by adding 1 to 5 per cent of dextrose to each soil in the tumblers.

4. Determine the influence of the mechanical and chemical composition of soils by using sand, sandy loams, loams, clays, and peaty soils.

5. Determine the influence of various nitrogenous substances such as, ground fish, meat meal, tankage, various green and dry manures, amino compounds, etc., adjusting the moisture content according to their absorbing powers. As these materials decompose slower than dried blood or cotton-seed meal, the incubation period must be extended.

6. Determine the influence of lime compounds, such as, calcium oxide, calcium carbonate or magnesium carbonate, making the additions at the rate of ½ to 1 per cent.

7. Determine the amount of ammonia produced in the decomposition of manures by the use of the aeration unit, (New York Agri. Exp. Sta. *Bull.*, 494, p. 7, 1922). The ammonia-free air is conducted over the sample and the freed ammonia is absorbed in standard acid solution and determined by titration. This determination may also include the determination of the carbon-dioxide produced by freeing the air of CO_2 and absorbing the amount produced in a suitable absorbent. (See, The production of carbon dioxide by microorganisms page 205.)

8. Determine the widespread ability of microorganisms to produce ammonia by inoculating either the solution or soil, prepared as described above and previously sterilized, with vigorous cultures of any of the common organisms and incubating at the optimum temperature of the organism.

BIOLOGICAL OXIDATION OF NITROGENOUS COMPOUNDS TO NITRITES AND NITRATES

While the production of ammonia may serve as a means of measuring biological activities in soils, most plants are unable to utilize the ammonia that is formed. Plants require their nitrogen in the form of nitrates, hence the ammonia must be oxidized by a relatively small group of bacteria that possesses this power. The action may be direct;

$$NH_3 + 2O_2 = HNO_3 + H_2O$$

or it may be in two stages;

$$NH_3 + 3O = HNO_2 + H_2O$$

$$HNO_2 + O = HNO_3.$$

The determination of nitrite formation, either in soil or in solution, is of little practical importance due to the fact that this form readily changes to the nitrate. It is of value, however, in demonstrating to the student the actual process of the reaction.

Determination of Nitrite Formation in Solution:

1. Prepare the following solution:

Winogradsky's Solution for Nitrite Formation

Ammonium sulfate ($(NH_4)_2 SO_4$)...	1.0 gram[1]
Di-potassium phosphate (K_2HPO_4)..	1.0 gram
Magnesium sulfate ($MgSO_4.7H_2O$)...	0.5 gram
Sodium chloride (NaCl).....	2.0 grams
Ferrous sulfate ($FeSO_4.7H_2O$).....	0.4 gram
Magnesium carbonate ($MgCO_3$)....	about 5.0 grams
Distilled water.....	1,000 cubic centimeters

[1] The ammonium sulfate is sterilized in a separate solution, as the magnesium carbonate may cause a loss of the ammonia during the sterilizing process. For this purpose a 5 or 10 per cent solution is made, sterilized separately and added to the other sterilized solution when cooled.

2. One hundred-cubic centimeter portions of the above solution, without the ammonium sulfate, are placed in 250-cubic centimeter Erlenmyer flasks, plugged and sterilized. The proper amount of ammonium sulfate solution, 1 cubic centimeter of a 10 per cent solution, is now added by means of a sterile pipette. The sterile solutions are next inoculated, in duplicate, with 10 cubic centimeters of a fresh soil infusion, and incubated for 7 to 8 days at room temperature.

3. Test qualitatively for the presence of nitrites (see, Nitrite Nitrogen, Tromsdorf's Test, Sec. 3, page 139).

4. A quantitative nitrite determination usually cannot be made at this stage, due to the fact that other organisms are utilizing or transforming the nitrites as fast as they are being formed. As this solution favors the growth of the nitrite forming organisms, the action may be intensified by transferring a loopful from the inoculated flask to a fresh sterile solution and again incubating. The second or third transfer should give enough material for a quantitative determination.

Determination of Nitrate Formation in Solution:

The formation of nitrates closely follows the formation of nitrites. Accordingly the same procedure is followed and the same solution used as directed for the determination of nitrite formation above, the only difference being in the time of incubation. In this case the period of incubation is 4 to 6 weeks. Thus the two determinations may be made on the same set of flasks.

At the end of the incubation period, add approximately 2 grams of precipitated calcium carbonate, or 2 cubic centimeters of

aluminum cream, to clarify the solution and filter an aliquot, or the entire solution, into a 250-cubic centimeter volumetric flask. Wash thoroughly with distilled water, make up to the mark, and determine the nitrates present in an aliquot by the regular phenol-disulfonic acid method, (see, Nitrate Nitrogen, Sec. 3, page 134).

Calculate the per cent of ammonia nitrogen oxidized to the nitrate.

If desired, the ability of the organisms to oxidize nitrites directly may be determined by substituting 1 gram of sodium nitrite for the ammonium sulfate in the above solution, using the same incubation period, 4 to 6 weeks. Calculate the per cent of nitrite nitrogen oxidized to the nitrate.

Determination of Nitrate Formation in Soils:

1. Sieve, mix thoroughly, and determine the optimum moisture content of the soils to be studied.

2. Weigh out in tumblers, six 100-gram portions of each soil. If the soils are not air-dry, the equivalent of 100 grams is to be used. Make additions as follows:

Tumblers	Additions
1 and 2.....	Nothing (check).
3 and 4.....	1 cubic centimeter of 10 per cent ammonium sulfate solution.
5 and 6.....	200 milligrams of dried blood (100 mesh or finer).

3. Mix thoroughly by means of a sterile spatula.

4. If air-dry soil has been used, inoculate each kind of soil with 10 cubic centimeters of its corresponding infusion.

5. Adjust the moisture content to optimum with sterile water. If moist soil has been used, the amount of moisture in it must be determined and allowed for.

6. Cover tumblers with metal covers to prevent evaporation. Weigh each tumbler and record the weight on the tumbler or cover. Incubate at room temperature for 4 to 6 weeks.

7. At the end of each week, weigh each tumbler and add sufficient sterile water to restore that lost by evaporation.

8. At the end of the incubation period, transfer the moist soil to shaker bottles, add 2 grams of precipitated chalk and sufficient distilled water to make a total of 500 cubic centimeters in the dry soil. For instance, if the soil contains 20 per cent (20 cubic centimeters) of moisture, the addition would be 480 cubic centimeters. Shake thoroughly for 20 minutes, filter, return the first 50 to 75 cubic centimeters to the filter, in order to obtain a per-

fectly clear extract, and determine the nitrates as directed under Nitrate Nitrogen, Sec. 3, page 134.

If not convenient to determine the nitrates at once, the moist samples must be quickly and thoroughly air dried. In the dry state there will be no change or loss of nitrates. Do not attempt to preserve with antiseptics.

Isolation of Nitrite and Nitrate-forming Organisms. *Enrichment Cultures.*—As indicated under Nitrite Formation in Solution, the activities of certain organisms may be markedly increased by repeated transfers to a sterile medium that favors their growth. This increase in activity is due primarily to an increase in numbers of the causative organisms. As the organisms possessing the desired characteristics are signally favored, they tend to outgrow other forms, and as more transfers are made, contamination is reduced to the minimum. Thus many transfers tend to increase or enrich the number of desired forms. Such a series of cultures are called Enrichment Cultures.

Unfortunately[10] very little is known of the biological forms possessing the power. They do not grow readily on plates and supposedly pure cultures may quickly lose their oxidizing powers. Apparently there is a relationship (possibly symbiotic), existing between the forms, that has not been worked out.

By using an extended series of enrichment cultures, it is possible to secure an impure culture that will give oxidation products in a very short time, 24 to 72 hours. The number of contaminating forms, appearing in the solution, are so few that for demonstration purposes it may be considered a pure culture.

Suggested Experiments

The nitrite and nitrate forming organisms cannot oxidize the nitrogen compounds in carbohydrate material until the ammonia forming organisms have first changed them to ammonia. As the activities of both groups of organisms are governed by the same factors, the suggested experiments (1 to 6 inclusive) under ammonia production, should be carried out. It must be remembered, however, that ammonia must be produced before nitrites and nitrates can be produced; consequently the incubation period should always be at least 3 weeks longer than the ammonia production period.

BIOLOGICAL REDUCTION OF NITROGENOUS COMPOUNDS

The Reduction of Nitrates. Practically all organisms require oxygen for their metabolic processes. If the oxygen supply is cut off, or is limited, the majority of the organisms die while others can secure their oxygen from compounds containing this element.

In the case of the nitrate radical, oxygen may be partially removed, thus forming the nitrite,

$$Ca(NO_3)_2 = Ca(NO_2)_2 + O_2$$

or it may be entirely removed and the nitrogen freed as a gas,

$$2Ca(NO_3)_2 = 2CaO + 2N_2 + 5O.$$

In the first case, the nitrogen is not lost, in the second it is lost completely. Little is known of the actual process; it is possible that many steps are involved, v.s.

$NO_3 \rightarrow N_2O_5 \rightarrow NO_2$ $N_2O_3 \rightarrow NO \rightarrow N_2O \rightarrow N_2 \rightarrow NH_2OH \rightarrow NH_3$.

Determination of Nitrate Reduction in Solution:

Prepare the following:

Giltay Solution

Di-potassium phosphate (K_2HPO_4)....	2.0 grams
Magnesium sulfate ($MgSO_4.7H_2O$)....	2.0 grams
Potassium nitrate (KNO_3).....	1.0 gram
Calcium chloride ($CaCl_2$. fused).....	0.2 gram
10 per cent ferric chloride (Fe_2Cl_6)....	2 drops
Citric acid crystals.....	5 grams
Distilled water.....	800 cubic centimeters

Dissolve the ingredients in the water, neutralize with 10 per cent sodium hydroxide, using phenolphthalein as indicator, adding just enough of the alkali to turn the solution a faint pink. Designate as solution 1.

Now dissolve 10 grams of dextrose in 200 cubic centimeters of distilled water. Designate as solution 2.

Add solution 1 to solution 2 and mix thoroughly.

1. Place 10 cubic centimeters of the above mixed solution in test tubes, plug and sterilize.

2. Inoculate in duplicate with approximately 5 cubic centimeters of soil infusion or with manure infusion, and incubate at room temperature.

3. At the end of each 24 hours, remove a few drops, using a sterile pipette or a sterile loop and test qualitatively for the presence of nitrates, using the di-phenylamine solution as directed in the test for nitrates, (see Nitrate Nitrogen, Sec. 3, page 133). Note the number of days required for the nitrates to disappear.

Isolation of Nitrate-reducing Organisms.—Make enrichment cultures in Giltay's solution as suggested under nitrate formation. After the third culture isolate on Giltay agar, (20 grams of agar added to 1,000 cubic centimeters of the above mixed solu-

tions), and test the colonies formed for their nitrate reducing powers. If the anaerobic organisms are to be isolated, place a film of paraffine oil (1 to 2 cubic centimeters) in the tubes containing the inoculated Giltay solution immediately after inoculation. Plate in the usual manner and place the plates under anaerobic conditions.

Determination of Nitrate Reduction in Soil:

1. Add 0.5 gram of calcium nitrate to 100-gram portions of air-dry soil. The nitrate is best added in the form of a 10 per cent solution. Inoculate the soil with 10 cubic centimeters of a fresh infusion, adjust the moisture content to the optimum, and incubate 14 to 21 days at room temperature.

2. At the end of the incubation period determine the amount of Nitrate Nitrogen (see, Sec. 3, page 134), present in a 50-gram portion of the different soils.

3. Place the remaining 50-gram portion on a filter, wash with distilled water until free of nitrates, dry the residue, grind, and determine the total nitrogen content by the regular Kjeldahl method, (see, Total Nitrogen, Sec. 3, page 127).

4. Calculate the amount of nitrogen lost.

Suggested Experiments

1. Determine the effects of large amounts (20 grams) of organic matter as dextrose, manures, etc.

2. Determine the influence of varying amounts and kinds of nitrate salts.

3. Determine the effect of greater amounts of moisture in stimulating nitrate reduction. Make the additions up to saturation.

BIOLOGICAL FIXATION OF ATMOSPHERIC NITROGEN

The fixation of atmospheric nitrogen is a function that is confined almost entirely to certain groups of bacteria. While little is known of the actual process it appears, first, that some form of assimilable carbon must be present to act as a source of energy; second, that the nitrogen is combined by the metabolic processes of the organism into a protein, hence the process may be considered an oxidative one. When the action takes place in the absence of plants, that is, when there is no mutual dependence existing between the organism and the plant, it is called **non-symbiotic.** When the action takes place in the presence of plants and there is a mutual dependence existing between the organism and the plant, it is called **symbiotic.**

Non-symbiotic Aerobic Fixation of Atmospheric Nitrogen, in Solution

1. Prepare the following solution:

Ashby's Solution

Mono-potassium phosphate (KH_2PO_4)	0.2 gram[1]
Mannit	15 grams
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 gram
Sodium chloride (NaCl)	0.2 gram
Calcium sulfate ($CaSO_4$)	0.1 gram
Calcium carbonate ($CaCO_3$)	5.0 grams
Distilled water	1,000 cubic centimeters

[1] Dissolve the mono-potassium phosphate in a portion of the distilled water, make neutral to phenolphthalein, using normal sodium hydroxide.

Dissolve the other ingredients in the balance of the water and mix thoroughly with the neutralized solution.

The use of dextrose or saccharose is permissible. For a solid medium use 15 grams of agar.

The medium recommended for the determination of the number of nitrogen fixing bacteria (*Azotobacter*), omitting the agar, may be used in place of the above.

2. Place 100-cubic centimeter portions of either of the above solutions in 500-cubic centimeter Erlenmyer flasks, plug and sterilize. Inoculate in duplicate with 10-cubic centimeter portions of fresh soil infusion and incubate for 10 to 20 days at room temperature. To prevent contamination in the check flasks, 20 cubic centimeters of concentrated sulfuric acid may be added at once.

3. At the end of the incubation period, transfer to Kjeldahl flasks, add the reagents for the determination of total nitrogen, place in the digestion rack, and boil down carefully to prevent foaming. When fumes are given off, increase the flame and digest until clear. Determine the total nitrogen present by the regular Kjeldahl method (see, Nitrogen, Sec. 3, page 127).

4. Subtract the total nitrogen in the soil infusion and in the solution, from that in the culture at the end of the incubation period. The difference represents that fixed by the organisms.

Isolation of Non-symbiotic, Aerobic, Nitrogen-fixing Bacteria (*Azotobacter*).—1. Prepare and inoculate additional flasks, as described above, incubating until a film is noticed forming on the surface of the liquid. Examine the film microscopically,

every other day, either by hanging drop or staining, for the presence of large-celled organisms. These organisms will be found enveloped in a gelatinous matrix and usually in clumps.

2. When the organisms appear in large numbers, separate them by diluting two to three loopfuls in 100 cubic centimeters of sterile water containing 10 to 20 grams of clean sand. Shake vigorously to break up the clumps and isolate by inoculating agar tubes of either of the above solutions and pouring plates. Test the resulting colonies for nitrogen-fixing powers.

Non-symbiotic Fixation of Atmospheric Nitrogen in Soil

1. Add 1- to 5-gram portions of dextrose or mannit to 100-gram portions of air-dry soil in tumblers and thoroughly mix with a sterile spatula. Inoculate with 10 cubic centimeters of the corresponding fresh soil infusion. Add sterile water to bring the moisture content up to the optimum, cover with metal covers and weigh, recording the weight on the covers. Incubate 2 to 3 weeks at room temperature.

2. Weigh at the end of each week and replace any moisture lost by evaporation.

3. At the end of the incubation period, dry thoroughly, grind, and analyze an aliquot, 10 to 15 grams, for total nitrogen, using the Kjeldahl method (see, Nitrogen, Sec. 3). The difference between the total nitrogen at the beginning and at the end of the experiment, gives the amount fixed in the soil.

Suggested Experiments

1. Determine the influence of the oxygen supply on nitrogen-fixation by increasing the surface area of the liquid solution. For instance, place 100 cubic centimeters of the solution in 1,000- or 2,000-cubic centimeter Erlenmyer flasks or in large Petri dishes. The opposite effect may be obtained by decreasing the surface area, using large tubes or small flasks.

2. Determine the nitrogen-fixing powers of pure cultures in solution or in sterilized soil.

3. Determine the effect of variations in culture medium by leaving out one or more of the components, sugar, phosphate, etc.

4. Determine the effect of acidity by partially neutralizing the solution, using one-fourth, one-half and three-fourth of the amount of sodium hydroxide required to neutralize. A more accurate determination is to adjust the medium to a definite P_H value, 6, 5.5, 5, 4.5 and 4.

5. Determine the effect of various kinds of sugars by substituting lactose, maltose, saccharose, etc., for dextrose or mannit.

6. Determine the influence of small amounts of available nitrates, on the growth and activities of the organisms in solution. For instance, add 0.1 to 20 milligrams of calcium nitrate to each 100 cubic centimeters of either of the above solutions before sterilizing.

Non-symbiotic Anaerobic Fixation of Atmospheric Nitrogen, in Solution

The predominating non-symbiotic nitrogen-fixing soil organism (*Clostridium*) is characterized by the production of resistant spores. This fact allows the organism to be easily separated from the majority of the other soil organisms by simply pasteurizing the soil. The organisms will grow well in the following solution:

Winogradsky's Solution for Anaerobic Nitrogen Fixation

Di-potassium phosphate (K_2HPO_4)	1.0 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 gram
Sodium chloride (NaCl)	0.01 gram
Ferrous sulfate ($FeSO_4.7H_2O$)	0.01 gram
Manganese sulfate ($MnSO_4.4H_2O$)	0.01 gram
Calcium carbonate ($CaCO_3$)	30.0 grams
Dextrose	20.0 grams
Distilled water	1,000 cubic centimeters

Pasteurized Soil Infusion.—Place 50 grams of fresh soil in a flask containing 200 cubic centimeters of sterile tap water. Heat slowly until the temperature of the soil solution is 80 to 85°C. and hold at this temperature for 15 minutes. Cool quickly and allow the particles to settle.

1. Place about 2 inches of the Winogradsky's solution in deep flasks or tubes, plug and sterilize. Inoculate with 10-cubic centimeter portions of the pasteurized soil infusion. If desired the solutions may be covered with a layer of paraffine oil, about ½ inch deep. Incubate 3 to 4 weeks at room temperature.

2. At the end of the incubation period determine the total nitrogen content of the solution as in the aerobic fixation experiments.

Isolation of Non-symbiotic, Anaerobic, Nitrogen-fixing Bacteria, (*Clostridium*).—Make transfers of the growths secured in the above solutions into sterile portions of the same solution, incubate under anaerobic conditions until a good growth is secured. Dilute in sterile tap water and inoculate a 2 per cent agar of the solution, incubating the plates under anaerobic conditions.

Symbiotic Fixation of Atmospheric Nitrogen.—An important source of nitrogen increase in soils is through the fixation of nitrogen by the symbiotic organism *Rhizobium leguminosarum* (*Bacterium, Pseudomonas,* or *Bacillus radiciola*) working in the nodules on the roots of legumes. The organism may be isolated from the soil on the medium for nitrogen fixers, (see, Determination of the Number of Bacteria, page 177). The process is lengthy as it involves the testing of the pure cultures for their ability to produce nodules. The organism may be quickly isolated in pure culture from the nodule.

Isolution of Rhizobium Leguminosarum from the Nodule.—1. Prepare a solid medium of either of the solutions used in the aerobic fixation of nitrogen experiment. If Ashby's agar is used, remove the excess of calcium carbonate by filtration.

2. Thoroughly wash the roots of several legumes that are well supplied with nodules. Carefully detach a nodule from the root and place in clean water. Keep the nodules from each species of legume separate. The nodules of soybeans or cowpeas are best for student manipulation as they are large and easily handled.

3. Sterilize the outside of the nodule by immersing in a 1 to 500 mercuric chloride solution for 3 to 5 minutes. The length of time will depend upon the size of the nodule. Wash six to eight times in sterile water to remove the mercuric chloride.

4. Wash in approximately 50 per cent alcohol, remove the alcohol by carefully flaming and place the nodule on a sterilized surface, *i.e.*, the flamed side of a bacteriological slide.

5. Cut open the nodule by means of a sterile knife or a chisel-edged sterile needle. Remove some of the inner contents and place in a few cubic centimeters of sterile water. In the case of small nodules, the entire nodule is placed in the sterile water and crushed. (A sterile solution of either of the above media may be used in place of the sterile water. In this case, the inoculated tubes are incubated a few days before proceeding further.)

6. Make loop transfers, from the water dilution or the inoculated solution, into sterile tubes of melted agar, cooled to 40°C. and pour plates in the usual manner. If desired the inoculation may be made directly from the nodule to the agar. Incubate the plates 6 to 8 days at room temperature or at 28°C. The *Rhizobium* colonies are characterized by a raised moist surface that is at first glistening and later changes to an opaque white. The sur-

face colonies are round and entire: the subsurface colonies are a peculiar lens shape. The colonies on the surface vary from 1½ to 4 millimeters in diameter, the subsurface colonies are about ½ millimeter until they reach the surface where they spread out into normal colonies.

7. Make stained preparations of the organisms.

8. At the same time the nodules are being prepared for the isolation of the organism, select a number of old, partially decayed nodules, wash and sterilize, then crush in a few drops of sterile water on a clean slide. Make smears of the milky solution, dry, fix, stain with aqueous fuchsin for ½ minute and examine for the presence of the involution forms. Save these slides for comparison with 7 above.

Suggested Experiments

1. Determine the nitrogen-fixing powers of the pure cultures in the same manner as directed for the aerobic non-symbiotic nitrogen-fixation.

2. Determine the nitrogen content of either *Azotobacter*, *Clostridium* or *Rhizobium* cells, by inoculating hard agar, 2 to 2½ per cent, in large Petri dishes and incubating. Scrape the mass off with a clean slide, dry, pulverize, and analyze in the usual manner. The total number of organisms in the material may be determined by diluting the mass to a definite volume and determining, using dilution methods, the number in an aliquot.

3. Determine the effect of additions of small amounts of nitrogen in stimulating the nitrogen fixing powers of the organisms as directed in Suggested Experiment 6, Non-symbiotic Nitrogen Fixation, page 199. Compare the effect of organic and inorganic forms of nitrogen.

4. Determine the ability of pure cultures of *Rhizobium* to form nodules as follows:

a. Place filter paper pulp moistened with Ashby's solution, or soft agar (0.7 to 1.0 per cent) in large tubes, 1 × 12 inches, and sterilize. The material should occupy the lower 2 inches of the tube.

b. Sterilize seeds with 1 to 500 mercuric chloride or with 50 per cent alcohol and wash well.

c. Drop the sterilized seeds in the tubes and inoculate with a suspension of a 48-hour culture of the organism isolated from the corresponding legume. Keep in a warm, light place but not in direct sunlight.

5. Prepare commercial cultures by slanting Ashby's solid medium in a bottle with flat sides and inoculating with 5 to 10 cubic centimeters of a vigorous culture of the organism. The use of a mother culture is recommended. Be sure that the inoculum covers the entire slant. When a vigorous growth is secured, flame the plug and seal the bottle by dipping the plug quickly in melted paraffine and replacing.

The mother culture is made by inoculating a sterile solution with a number (10 to 20) pure cultures, isolated from the same species of legume,

whose efficiency has been tested. This really amounts to an impure culture of the same strain of organisms. Such a culture apparently has more vigor than one prepared from a single organism.

6. Determine the cross inoculating power of the organisms by using the same methods as in 4, but inoculating the legume with organisms isolated from some other species of legume.[2]

7. Determine the effect of inoculation upon the growth and nitrogen content of legumes by growing the plants in sterile soil and inoculating with cultures isolated from the corresponding species and when mature, wash free of soil and analyze for total nitrogen. As tap water may contain some *Rhizobium*, the plants should be watered with distilled water.

8. Determine the effect of lime by adding ½ per cent of 20-mesh ground limestone to the soil as in 7 above.

BIOLOGICAL FIXATION OF CARBON

Practically all microorganisms require the presence of some form of carbon for their best growth. The majority of them decompose the carbon molecule into carbon dioxide and water. Certain forms of algae and a few bacteria, sulfur oxidizers, have the power of fixing atmospheric carbon without the presence of soluble carbohydrates. The action is unimportant in soils at the present time.

Fixation of Carbon by Algae.—1. Fill 200-cubic centimeter Erlenmyer flasks approximately two-thirds full with clean, dry sand that has been washed free of all organic matter, weigh the flasks carefully and inoculate with 10-cubic centimeter infusion of fresh soil. Adjust the moisture content to 60 per cent with either of the solutions used in the nitrogen fixing experiments, omitting the sugar and adding 0.5 gram of calcium or ammonium nitrate per 1,000 cubic centimeters. Plug loosely and keep in a light place. Note the appearance of a greenish growth on the sides of the flasks.

2. After 3 months, dry thoroughly and weigh. Ignite 50-gram portions and determine, by the Loss on Ignition method, page 40, the amount of carbon fixed.

Isolation of Algae.—The isolation of algae in pure culture is difficult, due to the fact that each species apparently has certain nutrient requirements that must be determined. The organisms are separated by dilution upon the same medium in which grown, and then grown in sterile sand, upon gypsum blocks, moist filter paper, moist porcelain plates, etc.

Suggested Experiments

1. Inoculate sterile sand with pure cultures and note carbon fixation.
2. After growth in 1 is started, inoculate with pure cultures of *Azotobacter*, incubate 8 to 10 weeks, and Kjeldahlize 10-gram portions. Note the effect of carbon fixation upon nitrogen-fixation.
3. Determine the different depths at which algae occur in the soil by inoculating with infusions made from samples taken at 6-inch intervals down to 4 feet in loams and clays and down to 6 to 8 feet in the more open soils.

BIOLOGICAL DECOMPOSITION OF CARBON

The destruction of carbon compounds by biological action is due to the utilization of the carbohydrates for the metabolic processes. In the process the carbon compounds are hydrolized and made soluble, carbon-dioxide is given off in large amounts, and many by-products are formed. The by-products may or may not be utilized according to the species of organism present. The action in soils is of great practical importance due to the fact that the liberated carbon-dioxide combines with water to form carbonic acid, which is a powerful solvent of the insoluble plant food materials. The action may be demonstrated with many kinds of carbohydrates but is best seen by the action on pure cellulose.

Decomposition of Cellulose in Solution.—1. Prepare the following solution:

McBeth and Scales Solution

Di-potassium phosphate (K_2HPO_4)..	1.0 gram
Ammonium sulfate ($(NH_4)_2SO_4$.....	2.0 grams
Magnesium sulfate ($MgSO_4.7H_2O$).	1.0 gram
Calcium carbonate ($CaCO_3$).....	2.0 grams
Sodium carbonate (Na_2CO_3).....	1.0 gram
Tap water.....	1,000.0 cubic centimeters

2. Place pieces of non-absorbent cotton or strips of filter paper, in large test tubes, cover with the above solution, plug and sterilize.
3. Inoculate in duplicate with either soil or manure infusions.
4. Cover duplicate tubes with a ½-inch layer of paraffine oil.
5. Note change in the insoluble carbohydrate at regular intervals.

Isolation of Cellulose Decomposing Organisms.—1. Prepare hydrated cellulose as a basis for the following agar:

Hydrated Cellulose.—Add 100 cubic centimeters of concentrated sulfuric acid to 60 cubic centimeters of distilled water and

allow to cool, place in a 2,000-cubic centimeter flask, and add 5 grams of filter paper, previously moistened and shake vigorously until the mass is dissolved. Fill the flask with ice-cold water, transfer to a Buchner or Gooch filter and wash until all traces of acid are removed. Reduce the volume of the filtrate to about 200 cubic centimeters, wash into a flask and build up to 800 cubic centimeters.

2. Prepare the following agar:

Scales Cellulose Agar

Solution 1

Dissolve 0.5 gram of peptone in 40 to 50 cubic centimeters of water and filter.

Solution 2

Di-potassium phosphate (K_2HPO_4)	0.2 gram
Potassium carbonate (K_2CO_3)	0.4 gram
Ferric sulfate ($Fe_2(SO_4)_3$)	0.02 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 gram
Calcium chloride ($CaCl_2$)	0.02 gram
Sodium chloride (NaCl)	0.002 gram
Hydrated cellulose	400.0 cubic centimeters

Mix solution 1 with solution 2, add sufficient distilled water to make 1,000 cubic centimeters and 15 grams of agar. Tube, plug, and sterilize in the usual manner.

3. Dilute a few loopfuls of the inoculated solutions, showing marked decomposing action, in the usual manner, and pour plates of the above agar. The cellulose decomposing organisms may be readily seen on the plates by the halo of dissolved cellulose surrounding them.*

Decomposition of Cellulose in Soil.—The decomposition of cellulose in soil may be demonstrated by placing strips of filter paper directly in the soil and keeping moist for several weeks. A better method is to place a layer of soil in Petri dishes and cover with a small circle of filter paper. If the soil is kept moist with the McBeth and Scales solution and the plates covered to prevent evaporation, the action will be readily observed.

HUMUS FORMATION

Humus is a result of cellulose or carbohydrate decomposition and is a complex polymerization or condensation product which

* For descriptions of the bacteria, see *Soil Science* 1, pp. 437–487, 1916.

has both hydroxyl and carboxyl groups. As a portion of the products are alkali soluble, these are taken as an index of humus formation. The presence of humus in soils has a marked influence on plant growth.

Humus Formation in Soil

1. Weigh out 100-gram portions of air-dry soil in tumblers and add 2- to 5-gram portions of finely ground wheat straw, oat straw, leguminous hays, or different manures, etc., and mix thoroughly.
2. Adjust to optimum moisture content, adding 2 cubic centimeters additional for each gram of organic matter added.
3. Incubate 2 to 4 months, keeping the moisture content at the optimum.
4. At the end of the incubation period dry the soil and determine humus as directed under Determination of Humus, Sec. 2, page 41.

THE PRODUCTION OF CARBON DIOXIDE BY MICROORGANISMS

The assimilation and decomposition of soluble carbohydrates is always accompanied by the production of carbon-dioxide. The rate of production may be taken as an indication of the activities of the organisms. The following has been suggested as some of the possible reactions involved in the complete destruction of glucose:

1. The glucose is broken down into alcohol and carbon-dioxide,

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

2. The alcohol may be oxidized to acetic acid and water,

$$C_2H_5OH + O_2 = CH_3COOH + H_2O.$$

3. The acetic acid may be broken down to methane and carbon-dioxide,

$$CH_3COOH = CH_4 + CO_2.$$

4. The methane may be oxidized to carbon-dioxide and water,

$$CH_4 + 2O_2 = CO_2 + H_2O.$$

Due to the process of assimilation by the organism, it is practically impossible to take a weighed amount of carbohydrate and account for all the products formed.

Carbon-dioxide Production in Solution.—The production of carbon-dioxide in solution may be determined by placing an inoculated carbohydrate solution in place of the soil as directed

below. Any of the suggested media containing carbohydrates may be used.

Carbon-dioxide Production in Soil

1. Mix the materials used for humus formation with a definite amount of soil. The production of carbon-dioxide from the soil itself, without the addition of any materials, may be determined.

2. Place 100 to 200 grams of the soil in 500-cubic centimeter suction flasks and adjust the moisture content to optimum.

3. Stopper the flasks with a one-holed stopper, fitted with a glass T, connect the flasks in series by attaching tubing to the arms of the Ts, close one end and connect the free end with a carbon-dioxide absorption tower.

4. Connect the arms of each flask with a cylinder filled approximately two-thirds full with $\frac{N}{1}$ sodium or potassium hydroxide. The connection is made by means of a long glass tube passing through a two-holed stopper and extending to the bottom of the cylinder, as described in the aeration apparatus, Sec. 3, page 53.

5. Stopper the cylinders and arrange a T, as in the case of the flasks, connecting the free end with the suction pump.

6. Arrange the suction so that air will be drawn slowly through the apparatus. Adjust the flow of air evenly for each flask by means of a screw clamp placed between the flasks and absorption cylinders, or by placing a small piece of thermometer tubing inside of the rubber connection.

7. Determine the amount of carbon-dioxide formed, every other day, by removing the absorption cylinder and measuring the CO_2 produced by the double titration method, (see, Titration of Carbon Dioxide, Sec. 3, page 119).

Suggested Experiments

1. Determine the influence of pure cultures of cellulose decomposing organisms on carbon-dioxide production using various forms of carbohydrate.

2. Determine the carbon-dioxide production in humus formation.

3. Determine the influence of nitrogen in stimulating carbon decomposition by using different forms of protein materials.

4. Determine the ability of the soil organisms to produce ammonia, as well as carbon-dioxide, from materials like dried blood, fresh horse manure, etc., by placing a tower containing $\frac{N}{20}$ acid in front of the carbon-dioxide absorption cylinder. Only a portion of the ammonia can be determined in this manner, due to absorption in the material itself, the balance must be

determined in the material in the usual manner, see, Ammonia, Sec. 3, page 96).

5. Determine the carbon-dioxide production from sterile soils by sterilizing a portion of the flasks used in the carbon-dioxide production in soils experiment. Empty, sterile flasks should be run as a check against leakage.

6. Determine the normal carbon-dioxide producing power of soils, either treated or untreated, by placing 1,000 grams of soil in 2,500-cubic centimeter bottles and using the same arrangement as in 5, (see, Laboratory Equipment, Aeration Unit Connected in Parallel, Sec. 3, page 55). All connections should be varnished or lacquered to prevent leakage.

BIOLOGICAL OXIDATION OF SULFUR

Sulfur, or its compounds, may be oxidized by biological action. In the process the sulfur may be used, in place of carbon compounds, as a source of energy. As some carbon is essential, it is obtained from the air or from inorganic carbonates. The causative organisms are divided into three primary groups[19] as follows:

1. Organisms oxidizing the sulfides with sulfur deposited within the cells of the organisms.

$$2H_2S + O_2 = 2H_2O + S_2.$$

2. Organisms oxidizing the thiosulfates to sulfates and persulfates, depositing the sulfur outside the cell.

$$2Na_2S_2O_3 + O_2 = 2Na_2SO_4 + 2S$$
$$3Na_2S_2O_3 + 5O = 2Na_2SO_4 + Na_2S_4O_6.$$

3. Organisms oxidizing sulfur with the production of acid.

$$2S + 2H_2O + 3O_2 = 2H_2SO_4.$$

The activities of the organisms in group 3 are of prime importance from the soil standpoint as the acid formed will react with insoluble phosphates to produce the soluble form. For reactions, see, Phosphorus, Sec. 3, page 149.

Oxidation of Sulfur in Solution

1. Prepare the following solution:

Waksman's Solution for Sulfur Oxidizers

Ammonium sulfate ($(NH_4)_2SO_4$)	0.2 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	0.5 gram
Calcium chloride ($CaCl_2$)	0.25 gram
Ferrous sulfate ($FeSO_4$)	trace
Mono-potassium phosphate (KH_2PO_4)	3.0 grams
Sulfur	10.0 grams
Distilled water	1,000.0 cubic centimeters

The sulfur should be reduced to a thin paste before adding to the solution. The P_H will be about 4.0.

2. Place 150-cubic centimeter portions of the solution in 250-cubic centimeter Erlenmyer flasks, plug and sterilize. Inoculate with 10 cubic centimeters of soil infusion and incubate 2 to 6 weeks at room temperature.

3. At the end of each week withdraw, by means of a sterile pipette, two or more, 5-cubic centimeter portions. Place one of the portions in a tube and determine the reaction P_H. Place the other portion in a 250-cubic centimeter flask, add approximately 85 cubic centimeters distilled water, enough 10 per cent barium chloride solution to precipitate all the sulfates, build up to 100 cubic centimeters and determine the amount of sulfur oxidized (see, Sulfur, Sec. 3, page 158).

Oxidation of Sulfur in Soil

1. Weigh out 100-gram portions of soil, place in tumblers and make additions as follows:

Tumbler	Additions
1 and 2	Nothing (check)
3 and 4	2 grams of sulfur
5 and 6	10 grams of sulfur

2. Adjust the moisture content to optimun, adding 2 cubic centimeters additional for each gram of sulfur added and incubate 4 to 6 weeks at room temperature. The tumblers should be weighed at the beginning of the experiment and at weekly intervals. Any loss due to evaporation should be compensated for.

3. At the end of the incubation period, determine the amount of sulfate sulfur present in a water extract of the soil (see, Sulfur, Sec. 3, page 160).

Effect of Sulfur Oxidation in Soil on Insoluble Phosphates

1. Prepare a *sulfur-floats-soil* compost as follows:

Air-dry soil	100 grams
Elemental sulfur	100 grams
Raw rock phosphate	400 grams

2. Weigh 100-gram portions into six tumblers, divide into three series and make additions as follows:

Series	Tumblers	Additions
1	1 and 2	Nothing (check)
2	3 and 4	Nothing (check)
3	5 and 6	2 grams of peptone

3. Inoculate with 10 cubic centimeters of a fresh soil infusion and incubate for 8 weeks at room temperature.

4. Treat each series as follows during the incubation period:

Series 1.—Effect of sulfur oxidation under laboratory conditions. Weigh tumblers each week and adjust moisture content.

Series 2.—Effect of aeration on sulfur oxidation. Thoroughly stir the contents of the tumblers each week and adjust the moisture content.

Series 3.—Effect of decomposable organic matter on sulfur oxidation. Treat as in series 1.

5. At the end of the incubation period, thoroughly air dry the compost, divide into 25-gram portions and determine the water-soluble and neutral-ammonium-citrate-soluble phosphorus and the water-soluble sulfur, (see Phosphorus and Sulfur, Sec. 3, pages 147 and 161).

Isolation of Sulfur Oxidizing Organisms

1. Make loop transfers from the flasks showing vigorous oxidation in the solution cultures to fresh sterile tubes of the same solution. This solution may also be inoculated with small particles of soil or the sulfur-floats-soil compost. When a good growth is secured make dilutions and pour plates of the following agar:

Waksman's Solid Medium for Sulfur Oxidizers

Sodium thiosulfate ($Na_2S_2O_3$)	5.0 grams
Ammonium chloride (NH_4Cl)	0.1 gram
Calcium chloride (CaCl)	0.25 gram
Mono-potassium phosphate (KH_2PO_4)	3.0 grams
Agar	20.0 grams
Distilled water	1,000.0 cubic centimeters

It frequently happens that a vigorous sulfur oxidation will be found in the Oxidation of Sulfur in Soil experiments, while very few bacteria may be found. In this case, pour plates and determine the number and kinds of fungi present. *Penicillium luteum*, a common soil fungus, has marked sulfur oxidizing powers.[1] This organism may be easily isolated on any acid medium suitable for the growth of fungi.

Suggested Experiments

1. Determine the effect of various inorganic sulfur compounds added to the soil.

2. Determine the effect of sulfur oxidation on soil nitrates by adding sulfur to soils previously treated with calcium, sodium, or potassium nitrate.[14]

$$5S + 6KNO_3 + 2CaCO_3 = 3K_2SO_4 + CaSO_4 + CO_2 + N_2.$$

3. Determine the effect of soluble carbohydrates, dextrose, saccharose, etc., upon sulfur oxidation, by making additions of 1 to 5 grams to the sulfur-floats-soil compost and treating in the usual manner.[16]

4. Determine the effect of sulfur on the conservation of nitrogen in manure by adding ½ gram of sulfur to 100 grams of fresh horse manure and incubating.[17]

BIOLOGICAL REDUCTION OF SULFATES

The reduction of sulfates takes place under anaerobic conditions.

Reduction of Sulfates in Solution

1. Prepare the following:

Van Delden's Solution for Sulfate Reduction

Di-potassium phosphate (K_2HPO_4)	0.5 gram
Magnesium sulfate ($MgSO_4.7H_2O$)	1.0 gram
Ferrous sulfate ($FeSO_4.7H_2O$)	trace
Asparagin	1.0 gram
Sodium lactate	5.0 grams
Tap water	1,000.0 cubic centimeters

2. Place in test tubes, plug, sterilize, and inoculate with small pieces of incubated soil from the sulfur oxidizing experiments, with manure or with sewage. Paraffine the plugs to make them airtight and incubate for 2 to 5 weeks at room temperature.

3. When the solution turns a dark-greenish color, remove plugs and test for hydrogen sulfide production by holding a strip of filter paper moistened with 10 per cent lead acetate solution over the end of the tube.

4. Compare the amount of sulfate sulfur in the check and the inoculated solutions.

Isolation of Sulfate-reducing Organisms. Prepare a gelatin medium by adding 150 grams of gelatin to the Van Delden's solution for sulfate reduction, tube, plug, and sterilize. Inoculate the melted sterilized gelatin with various dilutions from the inoculated tubes of solution that have shown a vigorous growth. Pour plates and incubate under anaerobic conditions. Test any black colonies for their sulfate-reducing powers.

DEMONSTRATION MATERIAL

The teacher frequently desires demonstrational material for class use, in order that he may illustrate certain points. In

addition to the suggestions given in the preceding pages the following suggestions are offered:

To demonstrate the production of acids by fungi.[4]

To demonstrate the dissolving effect of plant roots, with and without bacteria, upon rocks.[9]

To demonstrate the production of an active enzyme by fungi.[15]

To demonstrate the presence of enzymes in dried fungi.[6]

The biological determinations outlined in the preceding pages illustrate the four main chemical phases of biological soil activities, namely, *Digestion*, *Assimilation*, *Oxidation*, and *Reduction*.[8]

Due to the limited scope of biological investigations, the action is illustrated only for the elements Nitrogen, Carbon, Sulfur, and Phosphorus. It is quite possible that the soil contains organisms that have specific relationships to the many other elements. The field offers a fertile source of investigation for the biological student.

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MISCELLANEOUS TABLES AND FORMULA

International Atomic Weights, 1922

Aluminum	Al	27.0	Gold	Au	197.2
Antimony	Sb	120.2	Helium	He	4.00
Argon	A	39.9	Holmium	Ho	163.5
Arsenic	As	74.96	Hydrogen	H	1.008
Barium	Ba	137.37	Indium	In	114.8
Bismuth	Bi	209.0	Iodine	I	126.92
Boron	Bo	10.9	Iridium	Ir	193.1
Bromine	Br	79.92	Iron	Fe	55.84
Cadmium	Cd	112.40	Krypton	Kr	82.92
Calcium	Ca	40.07	Lanthanum	La	139.0
Carbon	C	12.005	Lead	Pb	207.2
Cerium	Ce	140.25	Lithium	Li	6.94
Cesium	Cs	132.81	Lutecium	Lu	175.0
Chlorine	Cl	35.46	Magnesium	Mg	24.32
Chromium	Cr	52.0	Manganese	Mn	54.93
Cobalt	Co	58.97	Mercury	Hg	200.6
Columbium	Cb	93.1	Molybdenum	Mo	96.0
Copper	Cu	63.57	Neodymium	Nd	144.3
Dysprosium	Dy	162.5	Neon	Ne	20.2
Erbium	Er	167.7	Nickel	Ni	58.68
Europium	Eu	152.0	Niton	Nt	222.4
Fluorine	F	19.0	Nitrogen	N	14.008
Gadolinum	Gd	157.3	Osmium	Os	190.9
Gallium	Ga	70.1	Oxygen	O	16.00
Germanium	Ge	72.5	Palladium	Pd	106.7
Glucinum	Gl	9.1	Phosphorus	P	31.04

Platinum	Pt	195.2	Tellurium	Te	127.5
Potassium	K	39.10	Terbium	Tb	159.2
Praseodymium	Pr	140.9	Thallium	Tl	204.0
Radium	Ra	226.0	Thorium	Th	232.15
Rhodium	Rh	102.9	Thulium	Tm	169.9
Rubidium	Rb	85.45	Tin	Sn	118.7
Ruthenium	Ru	101.7	Titanium	Ti	48.1
Samarium	Sa	150.4	Tungsten	W	184.0
Scandium	Sc	45.1	Uranium	U	238.2
Selenium	Se	79.2	Vanadium	V	51.0
Silicon	Si	28.1	Xenon	Xe	130.2
Silver	Ag	107.88	Ytterbium	Yb	173.5
Sodium	Na	23.00	Yttrium	Yt	89.33
Strontium	Sr	87.63	Zinc	Zn	65.37
Sulphur	S	32.06	Zirconium	Zr	90.6
Tantalum	Ta	181.5			

Specific Gravity of Solutions of Ammonia

(Temperature 15°C.) Lunge and Wiernik

Specific Gravity	Per Cent of Ammonia (NH_3)
0.990	2.31
0.980	4.80
0.970	7.31
0.960	9.91
0.950	12.74
0.940	15.63
0.930	18.64
0.920	21.75
0.918	22.39
0.916	23.03
0.914	23.68
0.912	24.33
0.910	24.99
0.908	25.65
0.906	26.31
0.904	26.98
0.902	27.65
0.900	28.33
0.898	29.01
0.896	29.69
0.894	30.37
0.892	31.05
0.890	31.75
0.888	32.50
0.886	33.25
0.884	34.10
0.882	34.95

Specific Gravity of Solutions of Hydrochloric, Nitric, and Sulfuric Acids

(Temperature 15°C.)

Specific gravity	Per cent by weight			Specific gravity	Per cent by weight	
	HCl	HNO_3	H_2SO_4		HNO_3	H_2SO_4
1.010	2.14	1.90	1.57	1.460	79.98	55.97
1.020	4.13	3.70	3.03	1.470	82.90	56.90
1.030	6.15	5.50	4.49	1.480	86.05	57.83
1.040	8.16	7.26	5.96	1.490	89.60	58.74
1.050	10.17	8.99	7.37	1.500	94.09	59.70
1.060	12.19	10.68	8.77	1.510	98.10	60.65
1.070	14.17	12.33	10.19	1.520	99.67	61.59
1.080	16.15	13.95	11.60	1.530		62.53
1.090	18.11	15.53	12.99	1.540		63.43
1.100	20.01	17.11	14.35	1.550		64.26
1.110	21.92	18.67	15.71	1.560		65.08
1.120	23.82	20.23	17.01	1.570		65.90
1.130	25.75	21.77	18.31	1.580		66.71
1.140	27.66	23.31	19.61	1.590		67.59
1.150	29.57	24.84	20.91	1.600		68.51
1.160	31.52	26.36	22.19	1.610		69.43
1.170	33.46	27.88	23.47	1.620		70.32
1.180	35.39	29.38	24.76	1.630		71.16
1.190	37.23	30.88	26.04	1.640		71.99
1.200	39.11	32.36	27.32	1.650		72.82
1.210		33.82	28.58	1.660		73.64
1.220		35.28	29.84	1.670		74.51
1.230		36.78	31.11	1.680		75.42
1.240		38.29	32.28	1.690		76.30
1.250		39.82	33.43	1.700		77.17
1.260		41.34	34.57	1.710		78.04
1.270		42.87	35.71	1.720		78.92
1.280		44.41	36.87	1.730		79.80
1.290		45.95	38.03	1.740		80.68
1.300		47.49	39.19	1.750		81.56
1.310		49.07	40.35	1.760		82.44
1.320		50.71	41.50	1.770		83.32
1.330		52.37	42.66	1.780		84.50
1.340		54.07	43.74	1.790		85.70
1.350		55.79	44.82	1.800		86.90
1.360		57.57	45.88	1.810		88.30
1.370		59.39	46.94	1.820		90.05
1.380		61.27	48.00	1.830		92.10
1.390		63.23	49.06	1.840		95.60
1.400		65.30	50.11	1.841		97.00
1.410		67.50	51.15	1.8415		97.70
1.420		69.80	52.15	1.841		98.20
1.430		72.17	53.11	1.840		99.20
1.440		74.68	54.07			
1.450		77.28	55.03			

Conversion Data

Temperature

Degrees Fahrenheit $- 32 \times \frac{5}{9}$ = degrees Centigrade
Degrees Centigrade $\times \frac{9}{5} + 32$ = degrees Fahrenheit

Length

1 millimeter = 0.03937 inch
1 Meter = { 39.37 inches / 3.28 feet

1 inch = 25.4001 millimeters
1 foot = 0.2048 meter

Area

144 square inches = 1 square foot
1,728 cubic inches = 1 cubic foot
43,560 square feet = 1 acre
Circumference of a circle = $2\pi R$ or πD
Area of a circle = πR^2
Area of a sphere = $4\pi R^2$ or πD^2

Capacity

1 cubic centimeter = 0.03381 liquid ounce
1 liquid ounce = 29.574 cubic centimeters
1 Liter (1,000 cubic centimeters) = 1.056 quarts or 61.02 cubic inches
Volume of a sphere = $\frac{4}{3}\pi R^3$ or $\frac{1}{6}\pi D^3$
Volume of a cylinder = $\pi R^2 H$ H = height

Mass

1 gram = 0.03527 ounces avoirdupois
1 ounce avoirdupois = 28.3495 grams
1 pound = { 453.59 grams / 0.45359 kilogram

1 kilogram = 2.204 pounds

MILLIGRAMS OF CARBON PER CUBIC CENTIMETER OF CARBON DIOXIDE AT DIFFERENT TEMPERATURES AND PRESSURES

$\frac{t}{p}$	720	722	724	726	728	730	732	734	736	738	740	742	744
10	.4851	.4864	.4878	.4891	.4905	.4919	.4933	.4947	.4960	.4974	.4987	.5001	.5014
11	.4829	.4842	.4856	.4869	.4883	.4896	.4910	.4924	.4937	.4951	.4964	.4978	.4991
12	.4806	.4819	.4833	.4846	.4860	.4873	.4887	.4901	.4914	.4928	.4941	.4955	.4968
13	.4783	.4796	.4810	.4823	.4837	.4850	.4864	.4878	.4891	.4905	.4918	.4932	.4945
14	.4760	.4773	.4787	.4800	.4814	.4827	.4841	.4855	.4868	.4882	.4895	.4908	.4921
15	.4737	.4750	.4764	.4777	.4791	.4804	.4818	.4832	.4845	.4858	.4871	.4884	.4897
16	.4714	.4727	.4741	.4754	.4768	.4781	.4795	.4808	.4821	.4834	.4847	.4860	.4873
17	.4691	.4704	.4718	.4731	.4745	.4758	.4771	.4784	.4797	.4810	.4823	.4836	.4849
18	.4668	.4681	.4694	.4707	.4721	.4734	.4747	.4760	.4773	.4786	.4799	.4812	.4825
19	.4644	.4657	.4670	.4683	.4697	.4710	.4723	.4736	.4749	.4762	.4775	.4788	.4801
20	.4620	.4633	.4646	.4660	.4673	.4686	.4699	.4712	.4725	.4738	.4751	.4764	.4777
21	.4596	.4609	.4622	.4636	.4649	.4662	.4675	.4688	.4701	.4714	.4727	.4740	.4753
22	.4572	.4585	.4598	.4612	.4625	.4638	.4651	.4664	.4677	.4690	.4703	.4716	.4729
23	.4548	.4561	.4574	.4587	.4600	.4613	.4626	.4639	.4652	.4665	.4678	.4691	.4704
24	.4523	.4536	.4549	.4562	.4575	.4588	.4601	.4614	.4627	.4640	.4653	.4666	.4679
25	.4498	.4511	.4524	.4537	.4550	.4563	.4576	.4589	.4602	.4614	.4627	.4640	.4653
26	.4473	.4486	.4499	.4512	.4524	.4537	.4550	.4563	.4576	.4588	.4601	.4614	.4627
27	.4447	.4460	.4473	.4486	.4498	.4511	.4524	.4537	.4550	.4562	.4575	.4588	.4601
28	.4421	.4434	.4447	.4460	.4472	.4485	.4498	.4511	.4524	.4536	.4549	.4562	.4575
29	.4395	.4408	.4420	.4433	.4445	.4458	.4471	.4484	.4497	.4509	.4522	.4535	.4548
30	.4368	.4381	.4393	.4406	.4418	.4431	.4444	.4457	.4470	.4482	.4495	.4508	.4521

$\frac{t}{p}$	746	748	750	752	754	756	758	760	762	764	766	768	770
10	.5028	.5041	.5055	.5069	.5083	.5096	.5110	.5124	.5137	.5151	.5165	.5178	.5192
11	.5005	.5018	.5032	.5046	.5060	.5073	.5087	.5101	.5114	.5127	.5141	.5154	.5168
12	.4982	.4995	.5009	.5023	.5036	.5049	.5063	.5077	.5090	.5103	.5117	.5130	.5144
13	.4959	.4972	.4986	.4999	.5012	.5025	.5039	.5053	.5066	.5079	.5093	.5106	.5120
14	.4935	.4948	.4962	.4975	.4988	.5001	.5015	.5029	.5042	.5055	.5069	.5082	.5096
15	.4911	.4924	.4938	.4951	.4964	.4977	.4991	.5005	.5018	.5031	.5045	.5058	.5072
16	.4887	.4900	.4914	.4927	.4940	.4953	.4967	.4981	.4994	.5007	.5021	.5034	.4048
17	.4863	.4876	.4890	.4903	.4916	.4929	.4943	.4957	.4970	.4983	.4997	.5010	.5024
18	.4849	.4852	.4866	.4879	.4892	.4905	.4919	.4933	.4946	.4959	.4973	.4986	.4999
19	.4815	.4828	.4842	.4855	.4868	.4881	.4895	.4908	.4921	.4934	.4948	.4961	.4974
20	.4791	.4804	.4818	.4831	.4844	.4857	.4870	.4883	.4896	.4909	.4923	.4936	.4949
21	.4767	.4780	.4793	.4806	.4819	.4832	.4845	.4858	.4871	.4884	.4898	.4911	.4924
22	.4742	.4755	.4768	.4781	.4794	.4807	.4820	.4833	.4846	.4859	.4873	.4886	.4899
23	.4717	.4730	.4743	.4756	.4769	.4782	.4795	.4808	.4821	.4834	.4847	.4860	.4873
24	.4692	.4705	.4718	.4731	.4744	.4757	.4770	.4783	.4795	.4808	.4821	.4834	.4847
25	.4666	.4679	.4692	.4705	.4718	.4731	.4744	.4757	.4769	.4782	.4795	.4808	.4821
26	.4640	.4653	.4666	.4679	.4692	.4705	.4718	.4731	.4743	.4756	.4769	.4782	.4795
27	.4614	.4627	.4639	.4652	.4665	.4678	.4691	.4704	.4716	.4729	.4742	.4755	.4768
28	.4587	.4600	.4612	.4625	.4638	.4651	.4664	.4677	.4689	.4702	.4715	.4728	.4741
29	.4560	.4573	.4585	.4598	.4611	.4624	.4637	.4650	.4662	.4675	.4688	.4701	.4714
30	.4533	.4546	.4558	.4571	.4584	.4597	.4610	.4623	.4635	.4647	.4660	.4673	.4686

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